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WHAT IS A GENE?—TWENTY YEARS LATER*

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Just a little more than twenty years ago—in 1933—I was asked to give a lecture in the series "Studies in Heredity" arranged by the Carnegie Institution of Washington. I chose the title "What is a Gene?" and attempted to present a picture of the gene as it appeared to me at that time. Now as this year's president of the American Society of Naturalists I am expected to deliver an address at this distinguished gathering. In selecting a topic I decided to turn once again to the question "What is a gene?"—and to describe the gene as I see it now, in the light of the great advances made in this field of research during the past twenty years.

It seems important to point out at the start of this discussion that all our knowledge about genes is derived from observation of the biological effects produced when certain changes occur in their action—changes that may be due either to modification in structure, to modification in position, or to the elimination of genes. So far, there is no way of studying a gene directly, by optical, physical, or biochemical means. Thus, in order to investigate the nature and properties of genes it is essential to be able to observe changes in their action. It is also important to be able to induce such changes experimentally—in order to increase the low frequency with which they ordinarily occur—and even more important to be able to vary and control the conditions responsible for them. Therefore Muller's development of a method for measuring mutation rates in Drosophila, and especially his discovery that ionizing radiation increases mutation rates, were among the most significant advances in genetics, because they provided geneticists with an important tool for the study of genic properties. During the past decade, methods have been devised for inducing mutations

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by means of various chemical treatments, and thus a new way has been opened up to investigate the conditions that bring about gene changes.

GENE MUTABILITY

Since mutability is of great importance in investigating the nature of genes, I shall take it up first in this discussion. The problems that I shall talk about—in connection with which we have obtained new experimental evidence—are concerned primarily with the induction of mutations by treatment with various mutagens. A considerable number of geneticists are working in this field, and significant results have been accumulated. I do not intend to present here a review of these results. It is my intention to deal only with evidence that is pertinent to our discussion, and to concentrate primarily on experimental findings obtained in our laboratory.

After Muller's discovery that mutations could be induced by ionizing radiations, a great deal of work was done in the study of dose-effect relationships, and elaborate hypotheses regarding single-hit effects and the size of a gene were evolved. Most of these studies, as well as some more recent studies of mutations induced by chemical agents, were made with *Drosophila melanogaster*, utilizing principles developed by Muller. The method is based on the observation of mutations in sex-linked genes which produce lethal effects. Since it appears probable that the sex-linked-lethal effect may be brought about through certain changes in any one of at least 300 genes located in the X chromosome of *D. melanogaster*, it is evident that this method measures the average frequency of a certain type of mutations occurring in a large number of genes. Because of the low rate of occurrence of both spontaneous and induced mutations, it is not possible in Drosophila to obtain adequate information about the behavior of individual genes for the purpose of quantitative analysis. Before I realized this situation, I made an attempt to study mutability at the "white" locus in order to test a hypothesis, outlined in the Carnegie Institution lecture referred to earlier (Demerec, 1933), about the constitution of the gene. I expected to observe changes from the wild type to various mutant alleles, and reversions to wild type; but after much labor I gave up the attempt, because I found no reversions whatever in a large number of experiments involving more than a hundred thousand flies.

The need for a setup in which it would be possible to study the mutability of single genes—and the evident unsuitability of Drosophila for this purpose—was my chief reason for turning, about twelve years ago, to the use of bacteria as material for my genetic studies. I hoped that methods for such studies could be developed with bacteria, because tremendous numbers of individuals can easily be handled in experiments. This hope has been fulfilled beyond my expectations. Using *Escherichia coli*, we have been successful in designing three sets of methods that make it possible to study mutability in an almost unlimited number of individual genes.

The first method that we have developed for the study of spontaneous and induced mutability in *E. coli* is concerned with mutations from sensi-

tivity to resistance to the T series of bacteriophages, the second with mutations from streptomycin dependence to non-dependence, and the third with mutations from deficiency (auxotrophy) to non-deficiency (prototrophy) with respect to certain nutrients essential for growth. In their operation these three methods utilize the same basic principle: the culturing conditions, in experiments using agar medium on Petri dishes, are so adjusted that only specific variants—certain mutant types—are able to grow and form colonies; and therefore the exact proportion of such a mutant type in a population of bacteria can be determined even when it is present in small numbers. Two important features characterize these methods: first, selection of the mutants is complete, so that the personal factor does not enter into the scoring; and, second, the experimental conditions can be regulated in such a way that the bacteria will pass through several divisions before selection begins, thus allowing the total number of induced mutants to become apparent.

TABLE 1

VALUES FOR FREQUENCY OF SPONTANEOUS REVERSION OBTAINED IN DIFFERENT EXPERIMENTS FOR FOUR GENES OF *ESCHERICHIA COLI*

Experiment	Mutants per 10^9			
	leu-1	ar-2	leu-6	try-6
1	0.08	0.34	0.83	4.50
2	0.12	0.20	1.59	7.35
3	0.05	0.34	1.52	7.00
4	0.08	0.40	1.74	7.52
5	0.04	0.22	1.09	4.66
6	0.08	0.50	2.17	4.12
7	0.06	0.44	1.67	5.30
8	0.06	0.53	1.53	4.45
9	0.06		0.68	5.61
Average	0.07	0.37	1.42	5.61
SE	±0.0078	±0.043	±0.16	±0.45

Using these methods, we are able to detect mutations that occur with a frequency of about 1×10^{-11}, and to obtain fairly accurate determinations of frequencies of 5×10^{-11} and higher. The data presented in table 1 illustrate the accuracy with which these determinations can be made. This table gives the results of independent experiments, carried out within a period of about one year, to determine spontaneous mutability to prototrophy in several auxotrophic strains.

As I have said, we are studying genetic changes involving resistance to phages, dependence on streptomycin, and nutritional deficiencies. Although, because of the nature of our material, we are not always able to ascertain whether or not we are dealing with changes affecting a single gene, we have good reason to believe that our observations involve changes in either one or a very few genes, because our mutants are similar to those found in other microorganisms, particularly Neurospora, in which the ge-

netic background of the mutability has been analyzed (Giles, 1951; Giles and Partridge, 1953).

In recent years we have been concentrating on comparative studies of the effects of treatment with mutagens on the mutability of various genes. In experiments using *E. coli* we have observed the behavior of about forty genes that control amino acid synthesis and streptomycin resistance. In each case the change studied was reverse mutation from a mutant type to the "wild type"—for example, leucineless → leucine$^+$, arginineless → arginine$^+$, tryptophaneless → tryptophane$^+$, streptomycin dependent → non-dependent. In the earlier experiments the mutagens used were ultraviolet radiation (UV), manganous chloride ($MnCl_2$), and beta-propiolactone (β-pl) (Demerec, 1953, 1954; Demerec *et al.*, 1954); and during the past winter S. W. Glover extended these tests to include X-rays, nitrogen mustard, diepoxybutane, and triethyleneimino triazine. With each mutagen used, the dosage was the same in all experiments—except for a few tests with UV and X-rays using the radiation-sensitive strain B—so that the results of treatments with any one mutagen are directly comparable. Since there is no way of determining what dose of a certain mutagen is equivalent to one of another mutagen, only indirect comparisons can be made of the effectiveness of different mutagens—by adjusting the treatments so as to leave similar proportions of survivors. In experiments with $MnCl_2$, however, which produces very little killing if any, we used the treatments that had been found to be most mutagenic (Demerec and Hanson, 1951).

TABLE 2

FREQUENCIES OF REVERSIONS PER 10^8 SURVIVING BACTERIA INDUCED IN VARIOUS GENES OF *ESCHERICHIA COLI* BY EITHER THE SAME TREATMENT OR SIMILAR TREATMENTS WITH $MnCl_2$ AND UV

Gene	Strain	$MnCl_2$	UV
phe-1	12-72	11	100
leu-5		594	57
ar-1	WP-12	52	510
try-5		14,700	3,110
leu-1	12-29	25	222
leu-2	M-1	24	1,200
try-2	M-4	448	10,700
ar-2	12-16	1,720	440
try-3	Sd-4-55	10,200	1,800

The results of these experiments clearly demonstrate the following features:

(1) *Gene specificity.* There is a high degree of specificity in the reaction of different genes to similar treatment with the same mutagen. Similar treatments—or the same treatment, when mutants of more than one type are selected in the same strain—may induce only a few mutations in some genes, more in others, and many in still others (table 2).

(2) *Mutagen specificity.* It has also been found that a gene which is strongly susceptible to the action of one mutagen may be only slightly af-

fected by another and react still differently to a third (table 2). If genes are arranged in order, according to frequency of induced mutants resulting from similar treatments with a certain mutagen, and this order is considered as representing the pattern of mutagenicity of that particular mutagen, the pattern will be found to be different for different mutagens (table 3).

TABLE 3

NINE GENES OF *ESCHERICHIA COLI* ARRANGED IN INCREASING ORDER OF MUTABILITY INDUCED BY TREATMENT WITH $MnCl_2$, UV, OR X-RAYS, SHOWING DIFFERENCES IN THE MUTAGENICITY PATTERNS OF THESE THREE MUTAGENS

$MnCl_2$		UV		X-rays	
Gene	Mutations per 10^8	Gene	Mutations per 10^8	Gene	Mutations per 10^8
phe-1	11	hi-1	22	leu-2	12
leu-2	24	phe-1	100	hi-1	34
ar-3	63	ar-2	440	ar-2	54
hi-1	121	leu-2	1,200	try-3	113
try-2	448	try-3	1,800	ar-3	468
leu-3	1,050	try-5	3,110	try-2	1,160
ar-2	1,720	ar-3	4,600	leu-3	1,380
try-3	10,200	leu-3	6,300	try-5	1,563
try-5	14,000	try-2	10,700	phe-1	2,460

(3) *Mutagen stability.* An unexpected outcome of our study was the finding that the mutability of certain genes could not be increased above the spontaneous level by any of the mutagens used in our experiments. Five such genes were found among 37 amino acid auxotrophs of *E. coli.* All these genes have the capacity to mutate, for they do so spontaneously, some with a high frequency and some with medium or low frequencies. But neither UV irradiation, X-ray irradiation, nor treatment with any of thirteen very potent chemical mutagens produced any detectable appearance of induced mutants. Studies now being made with a strain of *Salmonella typhimurium* indicate that the incidence of mutagen stability is considerably higher in that material than among the auxotrophic mutants of *E. coli.* Results obtained by Zlata Demerec (M. Demerec *et al.*, 1954) showed that one galactose-negative mutant out of eight, and seven serineless mutants out of fifteen, were mutagen stable. These results showed also that mutagen stability is a property of an allele rather than of a locus, for some alleles of the same locus were mutagen stable whereas others were not. Similarly, Szybalski's analysis (1954) of our *E. coli* material indicated that the mutagen-stable genes *hi-2* and *hi-3* in strains 12-23 and R-4-88, respectively, are allelic to *hi-1* of strain 12-91, which is not mutagen stable.

(4) *Specific mutagen stability.* Another interesting discovery, during our comparative study of the activity of various mutagens, was made by Glover (Demerec *et al.*, 1954), who found that some genes are stable with respect to certain mutagens, although other mutagens will increase their mutability. The results of Glover's experiments show that of all the mutagens tried

only X-rays and nitrogen mustard did not increase the mutability of *ar-1* (strain WP-12). Similarly, the mutability of *leu-2* (strain M-1) and *try-2* (strain M-4) was increased by all the mutagens except triazine.

(5) *Influence of genetic background.* Usually, genetic background does not modify the way in which a gene reacts to a mutagen, but occasionally such modification has been observed. A particularly good example was described by Glover (Demerec *et al.*, 1954). He observed that the effectiveness of UV, X-rays, and diepoxybutane in inducing reversions of *ar-3* was increased, and the effectiveness of triazine decreased, when *ar-3* was combined with *leu-3* instead of *leu-3*$^+$. Similarly, X-rays and nitrogen mustard increased the mutability of *ar-3* when it was combined with *try-2*, but not when it was combined with *try-2*$^+$. The gene *sd-4*, when in combination with *me-1*, is more susceptible to the mutagenic action of UV, X-rays, nitrogen mustard, diepoxybutane, and triazine than when it is combined with *me-1*$^+$, whereas the presence of either *cys-1*, *cys-2*, or *try-3* decreases its reaction to these mutagens.

(6) *Influence of environmental factors.* A very striking example of modification of mutagenic action by environmental factors was observed during studies of the mutagenicity of $MnCl_2$. It was found that the degree of effectiveness of that chemical as a mutagen was dependent on several factors that affect the physiology of the bacterial cells. In experiments using an *sd-4* strain (Demerec and Hanson, 1951), we found that the mutagenic effect of $MnCl_2$ was greater for bacteria grown in aerated cultures than for bacteria grown either in shaken or in non-aerated cultures, and also that it was more effective with resting than with growing bacteria. In addition, we observed that the results of treatment with this chemical were greatly influenced by the way the bacteria were handled before treatment—notably, what solution was used in washing them. For example, as compared with either unwashed cells or cells washed in 0.15 M NaCl, bacteria washed in 0.3 M NaCl showed greater induced mutability, those washed in 0.15 M $CaCl_2$ or $CoCl_2$ showed less, and those washed in either 0.15 M $MgCl_2$ or 0.15 M $ZnCl_2$ showed none at all.

(7) *Influence of post-treatment.* These studies of the mutagenic effect of $MnCl_2$ showed also that it can be modified by certain post-treatments (Demerec and Hanson, 1951). It was found that the induction of mutations was decreased if the bacteria were exposed to low temperature immediately after treatment. It was also decreased when the treated bacteria were washed in water, in 0.15 M NaCl, or in 0.15 M $MgCl_2$. Witkin (1953), working with phage-resistant mutants, and Berrie (1953), working with auxotrophs, found that when ultraviolet-irradiated bacteria were exposed to a temperature of 15°C after treatment the mutagenic effect of the irradiation was considerably less than when they were kept at 37°C. Furthermore, they observed that such post-treatment was effective only until the end of the first division cycle of the irradiated bacteria.

(8) *Delayed effect.* Another striking feature observed in our experiments was the delayed appearance of induced mutants. In work with phage re-

sistance it was found that some of the mutants induced by a mutagen appeared after the first division of the treated bacteria, that more appeared after the second, third, and subsequent divisions, and that it took between ten and twelve divisions for all the induced mutants to show up (Demerec, 1946). To analyze this behavior, a considerable amount of work has been done by Demerec (1953), Demerec and Cahn (1953), Labrum (1953), Newcombe (1953), Newcombe and Scott (1949), and Witkin (1951); and it seems probable that induced instability of a gene that persists through several cell divisions (a metastable condition) is the major mechanism responsible for the delayed effect. Analyses of auxotrophs in our collection have revealed the delayed effect in 17 out of 31 cases tested, or about 55 per cent. In the other cases, all induced mutants appeared after the first division of the treated bacteria.

Working hypothesis regarding gene mutability. As a preliminary interpretation of our observations, and as a help in planning further experiments, a working hypothesis has been formulated, which I shall outline briefly. It is assumed that the action of mutagens in inducing the mutations we are able to detect is indirect—in other words, that mutagenic treatment brings about some change in either cytosome or nucleus which in turn affects certain physiological (metabolic) processes of the cell and thereby influences genes. It is further assumed that different members of the gene complement (genome) react differently to the conditions in cells created by treatment with a mutagen. Some genes may be completely unaffected by most or even all mutagens (mutagen stability), others may be unaffected by certain mutagens (specific mutagen stability), whereas still others may be affected to various degrees. Apparently each member of the genome exhibits a specific type of reaction toward the same mutagen-created cell conditions. Since it has also been observed that genes react differently to treatment with different mutagens, it seems reasonable to assume that each mutagen is capable of inducing in cells a set of specific reactions. Spontaneous mutability, we can suppose, may be due to metabolic disturbances resulting, in occasional cells, from various unidentified causes. This assumption is supported by the findings of Bryson and Davidson (1951), who observed differences in the relative frequencies of several mutants when they occurred spontaneously and when they were induced by treatment with $MnCl_2$ or ultraviolet radiation. Such differences would be expected if gene mutability is stimulated by changes in the normal cell metabolism and if different changes are effected by different cell environments.

In brief, the evidence accumulated in experiments with ultraviolet radiation and manganous chloride indicates that the genetic effects of treatment with such agents may be modified—that is, increased or decreased—by subsequent exposure of the treated cells to certain other conditions, such as change of temperature. This modification may be accomplished only during the period before the end of first division of the treated cells, which

suggests that one division is sufficient to eliminate the effects of treatment and presumably to restore the cells' normal metabolic functioning.

GENE STRUCTURE

Allelism. In the lecture referred to earlier (Demerec, 1933) I cited evidence to suggest "that changes producing different allelomorphs are independent of each other and . . . that they might arise by changes in different groups of a gene molecule." The evidence referred to had been obtained in studies of unstable alleles at the "miniature" locus in *Drosophila virilis*. A plan to test this hypothesis was outlined in the paper; but the experiment, carried out later using the "white" locus in *D. melanogaster*, was not successful because, as mentioned earlier, it was not possible to obtain reverse mutations for a study of back-and-forth mutability.

A unique method for such a study of back-and-forth mutability became available about ten years later, when we investigated the problem of bacterial resistance to streptomycin. We found that one mutation in normally streptomycin-sensitive bacteria can give rise to mutants that are highly resistant to streptomycin. In strain B of *E. coli* about 60 per cent of such mutants, originating independently of one another, are not only resistant to streptomycin but also dependent on it for growth. By plating bacteria of a sensitive strain on a medium containing streptomycin, it is possible to isolate the resistant and dependent mutants, which alone are able to divide and form colonies; and by plating a streptomycin-dependent population onto medium lacking streptomycin one may select reverse mutants of the non-dependent and sensitive types. In both cases, very large numbers of bacteria can be used: for the study of mutations to resistance, about 1×10^9 per plate; and for the study of reversions, about 5×10^8 per plate. Repeated changes can be observed, from sensitivity to dependence and back to sensitivity, indefinitely. Several years ago (Demerec, 1950) we conducted such experiments and made a detailed analysis of 160 streptomycin-dependent mutant strains and 120 revertant strains. The following properties were studied: mutation rate, mutation pattern, growth rate, nutritional deficiency, sensitivity to ultraviolet radiation, and, in the case of the streptomycin-requiring mutants, whether or not the requirement could be satisfied by certain isolated parts of the streptomycin molecule.

Although we used only six out of many possible criteria to detect differences among them, we found that hardly any two of the mutants we studied were alike. This suggests that a reversion from dependence to sensitivity does not come about by a reversal of the chemical reaction that originally produced the dependence, but must be due to a change unlike the original one. It also suggests that there must be a considerable number of reactions that give rise to either streptomycin-resistant or streptomycin-sensitive mutants.

Since no techniques for producing recombination in strain B of *E. coli* were available at that time, a portion of this study was repeated with strain K-12, in order to determine whether recombination occurred between

the different resistant and dependent lines. We made intercrosses involving 12 dependent and 11 resistant lines, without observing any recombinants. Similar results were obtained by Newcombe and Nyholm (1950), who tested nine resistant and four dependent lines of K-12. These findings indicate that streptomycin resistance and dependence are inherited as though controlled by alleles either at one gene locus or at a very few loci. Now, if it is true that no two streptomycin-resistance mutants are alike, and also that many such mutants are allelic, then it follows that a different change at the gene locus is responsible for each newly arising mutant.

That is as far as we were able to proceed with the material then available. Studies with Salmonella, however, which I am going to discuss next, have opened up new possibilities for a further investigation of the relationships among alleles at the very interesting locus or loci determining streptomycin resistance.

Pseudoallelism. A serious drawback in using strain B of *E. coli* for the study of mutability was the fact that methods were not available for making standard genetic analyses in this material—such as a determination of whether or not similar mutants are allelic. For this reason we tried to work with Lederberg's strain K-12, but found it technically unsuitable for our purposes. Methods have recently been developed for crossing strains B and K-12 (reports by E. Calef and by W. Szybalski and P. D. Skaar in *Microbial Genetics Bulletin*, No. 9, January, 1954), and these should be very helpful in our work. Before I was aware of this new development, however, we started to experiment with *Salmonella typhimurium*, because Zinder and Lederberg (1952) had shown that transduction can be used to determine allelic relationships, and also because from the technical standpoint Salmonella is very suitable for our studies. Our original plan was to repeat with Salmonella experiments we had already carried out with *E. coli* to compare the action of various mutagens, using auxotrophs deficient in amino acid synthesis. In addition, it was planned to extend that work to include auxotrophs deficient in the synthesis of purines and pyrimidines and in the fermentation of sugars, and to use transduction as a means of determining which of a series of mutants exhibiting the same property were allelic and which were due to mutations occurring at different loci. As the first step in our work, we obtained a large number of independently originating auxotrophs. Our collection now includes 40 strains deficient for cystine, 27 deficient for histidine, 11 for isoleucine, 11 for leucine, 15 for methionine, 22 for proline, 29 for serine, 11 for tryptophane, 13 for adenine, and 12 for adenine plus thiamine, as well as 46 strains that are unable to ferment galactose. Next, we proceeded with tests for allelism by preparing cultures of temperate phages and using them in transduction experiments. It has been shown by Zinder and Lederberg (1952) that phage raised on wild-type bacteria is able to induce changes to wild-type in a small fraction (one per 10^5 to 10^6) of mutant bacteria, but that phage raised on mutant bacteria cannot transduce another population of mutant bacteria if the mutants are allelic. Extensive analysis of the transduction phenomenon

suggests, as the most probable mechanism, that a phage particle picks up a segment of a chromosome belonging to the bacterium in which it originates, and, when it infects another bacterium, deposits this segment within it. Presumably, the segment then synapses with the homologous section of the chromosome of the host bacterium and during the divisions of that bacterium is in some way incorporated into the chromosome of one of its progeny.

Transduction tests involving about 40 cystineless mutants (*cys-1*, *cys-2*, etc.) gave very interesting results. In these tests no transduction was observed between *cys-20* and *cys-1*, *-3*, *-5*, *-13*, *-21*, and *-22*, indicating

TABLE 4

NUMBERS OF TRANSDUCTIONS OBSERVED IN EXPERIMENTS WITH *SALMONELLA TYPHIMURIUM* IN WHICH ABOUT 1 × 10^8 BACTERIA AND 1 × 10^9 PHAGE PARTICLES WERE USED IN EACH COMBINATION

Bacteria	Phages raised on							Wild
	cys-1	cys-3	cys-5	cys-13	cys-20	cys-21	cys-22	
cys-1	0	4	71	3	0	28	24	664
-3	73	0	0	17	0	106	71	723
-5	36	0	0	12	0	51	67	502
-13	11	2	24	0	0	20	21	280
-20	0	0	0	0	0	0	0	254
-21	4	1	12	4	0	0	11	326
-22	54	26	212	28	0	117	0	484
-23	1707	464	1896	822	1114	1115	907	979

that these last six are allelic to *cys-20*. There was also no transduction between *cys-3* and *cys-5*, indicating allelism between them. Transduction occurred between all the other members of the group *cys-1*, *-3*, *-5*, *-13*, *-20*, *-21*, and *-22* (group A); but the number of transductions was considerably smaller than the number occurring either when wild-type phage from other *cys* strains was used with bacteria of group A or when other *cys* bacteria were used with group-A phage (table 4). A similar relationship was found among *cys-10*, *-12*, *-14*, *-15*, *-16*, *-18*, *-24*, *-25*, *-27*, *-40*, and *-41* (group B); among *cys-7*, *-23*, *-28*, *-29*, *-36*, *-37*, *-38*, and *-42* (group C); and among *cys-2*, *-6*, *-8*, *-17*, and *-39* (group D). Tests indicated that in members of group A the cystine deficiency may be partially satisfied by cystathionine, and that it may be partially satisfied in members of group C by either methionine, homocysteine, homoserine, or cystathionine.

The same general situation obtains with regard to all the other auxotrophic mutants that have been analyzed. Similar auxotrophs can be separated by transduction tests into well-defined groups; and this grouping coincides with the grouping that has been obtain d by biochemical investigations of blocks in the chain of reactions leading to the synthesis of the compound required by the auxotrophs. For example: Transduction experiments with

ten tryptophaneless mutants placed them in four groups—A, *try-8*; B, *try-2*, *-4*; C, *try-3*; and D, *try-1*, *-6*, *-7*, *-9*, *-10*, and *-11*. An analysis made by Dr. Sydney Brenner showed that the deficiencies of these four groups of auxotrophs involved four different reactions in the chain of synthesis of tryptophane, a situation identical to that he had previously analyzed with mutants of *E. coli* (in manuscript). The group-A mutant failed to synthesize anthranilic acid; the group-B mutants were unable to convert anthranilic acid to an as yet unidentified intermediate, called "compound B"; the group-C type failed to convert compound B to indole; and those of group D were blocked in the conversion of indole to tryptophane.

Our 27 histidineless mutants fall, according to transduction tests, into seven groups, which coincide with seven blocks in the synthesis of histidine. Twelve adenineless mutants fall into three groups, coinciding with three biochemical blocks. Further tests have divided 16 prolineless mutants into two groups, 21 serineless mutants into two groups, and 10 galactose-negative mutants into two groups.

These results favor the assumption that members of each such group are allelic to one another, and that the occurrence of a small amount of transduction within a group can be explained on much the same basis as the infrequent recombination that takes place between pseudoalleles. They indicate that a gene locus comprises a section of a chromosome, and that mutational changes occurring in different regions of this section give rise to different alleles. They also indicate that regions of the section may separate, and recombine with homologous regions within a locus of another chromosome.

DISCUSSION AND CONCLUSIONS

Two aspects of the gene problem—gene structure and gene mutability—are being considered in this presentation, and I shall concentrate my discussion on them. I shall try to project the changing picture of the gene that seems to be emerging from current studies of its biological reactions, and shall discuss the conditions that may be responsible for gene changes.

To avoid misunderstandings—particularly since there seems to be some uncertainty about their use—I shall first define the terms "gene" and "locus" as I am employing them. The term "locus" was introduced by Morgan's group, to indicate the place in a chromosome where a gene is located. (Morgan, Sturtevant, Muller, and Bridges, 1915, page 155; Morgan, 1919, page 251; Bridges and Brehme, 1944, page 2.) The necessity for such a term became evident after the discovery of multiple alleles. Thus in the case of the genes responsible for white eye color in Drosophila and its alleles "apricot," "eosin," "blood," and so forth, the position on the chromosome occupied by any one of these genes was designated as the "white locus." There seems to be little doubt about the original meaning of the term "locus," namely, a specific region of a chromosome. In that case the term "gene" applies to a unit of inheritance which occupies a locus. I am using the terms "locus" and "gene" in this sense.

An important contribution to the understanding of gene structure was the discovery of crossing over between multiple alleles, a phenomenon to which the name "pseudoallelism" has been applied. Such behavior has been observed in Drosophila (Lewis, 1945, 1951; Green and Green, 1949; Green, 1954; MacKendrick and Pontecorvo, 1952), maize (Laughnan, 1949), cotton (Stephens, 1951), Aspergillus (Roper, 1950; Pontecorvo, 1953), and Neurospora (Giles, 1951). This discovery revealed a striking feature—that the potentiality for the occurrence of the modifications responsible for the appearance of allelic mutants is present in many parts of the segment of a chromosome which constitutes a locus, and that the regions within the locus where these modifications occur can be recombined by crossing over, showing that they are arranged linearly along the chromosome.

Up to now, relatively few cases of pseudoallelism have been established, doubtless because of the complex and laborious techniques involved in analyzing them. However, since it appears that crossing over within a locus—that is, the ability of different regions to recombine—has been detected whenever a special search for it has been made, it seems probable that this separability of regions of a locus is a general characteristic of loci rather than a specific property of certain ones.

This is supported by evidence now accumulating in our work with Salmonella. We have demonstrated the existence of two or more alleles at 15 loci, by means of the transduction tests mentioned earlier. Among a total of 75 mutants that have been adequately tested—and with 220 chances of finding no recombinants—we have found only eight cases in which there was absence of recombination with one other allele of the same locus, and only one case (*cys-20*) of failure to recombine with any of the alleles of the locus. Absence of recombinants may be due to any of the following three circumstances: the occurrence of mutations at the same position in two alleles; the occurrence of mutations at two positions that do not participate in crossing over, either because of too close proximity or because of the structure of the region; or the presence of some chromosomal abnormality, such as inversion, which prevents recombination. The evidence we have available at present suggests that the non-appearance of recombinants is exceptional rather than common.

A considerable part of the evidence on which my conclusions about gene structure are based has been obtained in our work with Salmonella, which has not yet been published. The experiments essential for these conclusions have been completed, but I feel that this is not the occasion for a detailed presentation of complex data. That will be reserved for another paper. Most of the evidence was obtained in studies, already described, of the relations between independent mutants of similar phenotype, such as the series of cystineless mutants discussed earlier. These studies revealed allelic groupings, indicated both by transduction tests and by the analysis of blocks in the chain of synthesis of the compounds required by the mutants. A second important source of evidence was a study of transductions

involving three adjacent loci—*tryA*, *cysB*, and *tryD*—in which one, eleven, and six alleles, respectively, were available for use as markers. All the data we now have support the assumption that crossing over between a chromosome of the recipient bacterium and a segment of chromosome brought by transducing phage from a donor bacterium is responsible for transduction.

I believe that it is much too early to speculate about the chemical structure of genes, or about the nature of the genic changes that are responsible for the appearance of various alleles. I only wish to point out that the chemical structure of deoxyribonucleic acid postulated by Watson and Crick (1953) would account for the events at a locus which are detectable by genetic methods.

The other phase of our problem, gene mutability, was considered more fully in the first section of this paper and will be dealt with rather briefly here. The experimental evidence now available, from our studies and those of others, indicates that mutagens affect genes indirectly, through changes they induce in either the cytosome or the nucleus of a cell. Apparently the stability of genes—which is very probably a function of their power of exact self-duplication—is affected by their immediate environment. A striking feature brought out by our experiments is the tremendous variation in the reaction of different genes to treatment of cells by the same mutagen. Some genes are unaffected by any mutagen (mutagen stable), some are affected by certain mutagens but not by certain others, and some are affected to various degrees by different mutagens. Since it has been shown that certain alleles of the same locus may be mutagen stable whereas others are not, and also that the same gene may be stable (with regard to a certain mutagen) when it is a member of one genotype but not when it is a member of another genotype, it seems likely that mutagen stability results from a gene's ability to find in its immediate environment all the components necessary for reproducing as an exact replica, rather than from any inherent stability of chemical structure of the gene itself.

Finally, I wish to highlight the observation that both the spontaneous and the induced mutability of a gene may be influenced by the presence of other genes in the same cell. A good example of such influence was demonstrated by Glover (Demerec *et al.*, 1954) in experiments with *E. coli.* He showed that the frequency of reverse mutations induced in the gene *sd-4* (streptomycin dependence) by treatment of bacteria with either UV, X-rays, nitrogen mustard, diepoxybutane, or triazine was higher in the *me-1 sd-4* genotype than in the *sd-4* genotype, and was lower in the combinations *cys-1 sd-4*, *cys-2 sd-4*, and *try-3 sd-4*. Many examples could be cited of the influence of genetic factors on rate of spontaneous mutation. I shall mention here the mutability-stimulating factors observed in Drosophila (Demerec, 1937; Plough and Holthausen, 1937; Neel, 1942; Ives, 1950). In such cases the presence of a certain gene increases the mutability of the whole genome. Other examples of this type of effect include the modifier genes that influence the mutability of other specific genes—like the *s*

genes, which alter the rate of mutation of the unstable factor *mt* in *D. virilis* (Demerec, 1929), and the *Dt* gene, which determines the degree of mutability of A_1 in maize (Rhoades, 1938). The very interesting cases of gene instability brought about through the presence and proximity of the *Ds* and *Ac* factors in maize belong in this class of effects (McClintock, 1951, 1953). Also included here are those cases in which the stability of a gene, or even of a series of genes located in a particular region of a chromosome, is affected by the presence of another segment of chromosomal complex introduced by translocation, inversion, or transposition. It has been observed in Drosophila that when genes located in euchromatic regions of a chromosome are brought into proximity with certain heterochromatic regions these genes become unstable, and that the effect may extend throughout a section equivalent to about fifty bands on the salivary-gland chromosome map (Demerec, 1940, 1941; Kaufmann, 1942).

SUMMARY

I shall conclude with a brief summary of my present views about the gene. I regard a gene as a segment of a larger filamentous structure (chromosome) which is a center of specific biological activity and which is sufficiently different, chemically, from adjacent segments (other genes) as to possess different activity. I think of a gene as having three important properties: the capacity to reproduce itself; the capacity to produce a specific substance, which is responsible for its physiological activity; and the ability, during reproduction, to synapse with a homologous gene. It seems reasonable to assume that a gene reproduces by attracting from its cellular environment the materials of which it is constructed, and making it possible for them to join together. When two synapsed genes are reproducing, it may be supposed that each of them attracts its own set of compounds, and that components accumulated by either of them may be used in the formation of two new genes—a process similar to that proposed by Belling (1933) for the reproduction of chromosomes. In the course of such gene reproduction one may visualize various possible "failures," such as the substitution of one component for another, or a shift in the order of components, which would give rise to a slightly modified gene—that is, an allele of the original one. In the reproduction of two synapsed alleles, one having a failure in one region and the other a failure in another region, a newly formed gene might acquire one, both, or neither of these regions, and consequently there might occur recombinations of the characteristics of the original alleles (pseudoallelism).

According to this concept, gene changes (mutations) occur during gene reproduction. It is postulated that agents which increase mutability—such as mutagenic radiations and chemicals, mutator genes, specific chromosomal rearrangements—do so by creating conditions in treated cells that increase the frequency with which the genes fail to reproduce as exact replicas of themselves.

LITERATURE CITED

Belling, J., 1933, Crossing over and gene rearrangement in flowering plants. Genetics 18: 388–413.

Berrie, A. M. M., 1953, The effects of temperature on ultraviolet-induced mutability in *Escherichia coli*. Proc. Nat. Acad. Sci. 39: 1125–1133.

Bridges, C. B., and K. S. Brehme, 1944, The mutants of *Drosophila melanogaster*. Carnegie Institution of Washington Publ. 552.

Bryson, V., and H. Davidson, 1951, Spontaneous and ultra-violet-induced mutations to phage resistance in *Escherichia coli*. Proc. Nat. Acad. Sci. 37: 784–791.

Demerec, M., 1929, Genetic factors stimulating mutability of the miniature-gamma wing character of *Drosophila virilis*. Proc. Nat. Acad. Sci. 15: 834–838.

1937, Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics 22: 469–478.

1940, Genetic behavior of euchromatic segments inserted into heterochromatin. Genetics 25: 618–627.

1941, The nature of changes in the white-Notch region of the X-chromosome of *Drosophila melanogaster*. Proc. 7th Internat. Congr. of Genetics: 99–103.

1946, Induced mutations and possible mechanisms of the transmission of heredity in *Escherichia coli*. Proc. Nat. Acad. Sci. 32: 36–46.

1950, Reaction of populations of unicellular organisms to extreme changes in environment. Amer. Nat. 84: 5–16.

1953, Reaction of genes of *Escherichia coli* to certain mutagens. Symp. Soc. Exp. Biol. 7: 43–54.

1954, Genetic action of mutagens. Proc. 9th Internat. Congr. of Genetics:

Demerec, M., and E. Cahn, 1953, Studies of mutability in nutritionally deficient strains of *Escherichia coli*. I. Genetic analysis of five auxotrophic strains. J. Bacteriology 65: 27–36.

Demerec, M., and J. Hanson, 1951, Mutagenic action of manganous chloride. Cold Spring Harbor Symp. Quant. Biol. 16: 215–228.

Demerec, M., E. M. Witkin, H. Moser, J. Hemmerly, I. Blomstrand, Z. Demerec, P. FitzGerald, S. W. Glover, J. Hanson, A. M. Lacy, F. J. Nielsen, and T. Yura, 1954, Bacterial genetics. Carnegie Inst. Wash. Year Book No. 53: 225–241.

Giles, N. H., 1951, Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. Cold Spring Harbor Symp. Quant. Biol. 16: 283–313.

Giles, N. H., and C. W. H. Partridge, 1953, The effect of a suppressor on allelic inositolless mutants in *Neurospora crassa*. Proc. Nat. Acad. Sci. 39: 479–488.

Green, M. M., 1954, Pseudoallelism at the vermilion locus in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 40: 92–99.

Green, M. M., and K. C. Green, 1949, Crossing-over between alleles at the lozenge locus in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. 35: 586–591.

Ives, P. T., 1950, The importance of mutation rate genes in evolution. Evolution 4: 236–252.

Kaufmann, B. P., 1942, Reversion from roughest to wild type in *Drosophila melanogaster*. Genetics 27: 537–549.

Labrum, E. L., 1953, The effect of generation time on the delayed appearance of induced mutants in *Escherichia coli*. Proc. Nat. Acad. Sci. 39: 1221–1227.

Laughnan, J. R., 1949, The action of allelic forms of the gene A in maize. II. The relation of crossing over to mutation of A^b. Proc. Nat. Acad. Sci. 35: 167–178.

Lewis, E. B., 1945, The relation of repeats to position effects in *Drosophila melanogaster*. Genetics 30: 137–166.

1951, Pseudoallelism and gene evolution. Cold Spring Harbor Symp. Quant. Biol. 16: 159–174.

Luria, S. E., and M. Delbrück, 1943, Mutations of bacteria from virus sensitivity to virus resistance. Genetics 28: 491–511.

McClintock, B., 1951, Chromosome organization of genic expression. Cold Spring Harbor Symp. Quant. Biol. 16: 13–47.

1953, Mutation in maize. Carnegie Inst. Wash. Year Book No. 52: 227–237.

MacKendrick, M. E., 1953, Further examples of crossing over between alleles of the *w* series. Drosophila Information Service No. 27: 100.

MacKendrick, M. E., and G. Pontecorvo, 1952, Crossing-over between alleles at the *w* locus in *Drosophila melanogaster*. Experientia 8: 390–391.

Morgan, T. H., 1919, The physical basis of heredity. Philadelphia: J. B. Lippincott Company.

Morgan, T. H., C. B. Bridges, and A. H. Sturtevant, 1925, The genetics of Drosophila. Bibliogr. Gen. 2: 1–262.

Morgan, T. H., A. H. Sturtevant, H. J. Muller, and C. B. Bridges, 1915, The mechanism of mendelian heredity. New York: Henry Holt and Company.

Neel, J. V., 1942, A study of a case of high mutation rate in *Drosophila melanogaster*. Genetics 27: 519–536.

Newcombe, H. B., 1953, The delayed appearance of radiation-induced genetic changes in bacteria. Genetics 38: 134–151.

Newcombe, H. B., and M. H. Nyholm, 1950, The inheritance of streptomycin resistance and dependence in crosses of *Escherichia coli*. Genetics 35: 603–611.

Newcombe, H. B., and G. W. Scott, 1949, Factors responsible for the delayed appearance of radiation-induced mutations in *Escherichia coli*. Genetics 34: 475–492.

Plough, H. H., and C. F. Holthausen, 1937, A case of high mutation frequency without environmental change. Amer. Nat. 71: 185–187.

Pontecorvo, G., 1953, The genetics of *Aspergillus nidulans*. Advances in Genetics 5: 141–238.

Rhoades, M. M., 1938, Effect of the *Dt* gene on the mutability of the a_1 allele in maize. Genetics 23: 377–397.

Roper, J. A., 1950, Search for linkage between genes determining a vitamin requirement. Nature 166: 956–957.

Stephens, S., G., 1951, "Homologous" genetic loci in Gossypium. Cold Spring Harbor Symp. Quant. Biol. 16: 131–141.

Szybalski, W., 1954, Bacterial genetics and action of antimicrobial agents. Ann. Rpt. Biol. Lab. 1953–1954: 27–31.

Watson, J. D., and F. H. Crick, 1953, The structure of DNA. Cold Spring Harbor Symp. Quant. Biol. 18: 123–131.

Witkin, E. M., 1951, Nuclear segregation and the delayed appearance of induced mutants in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 16: 357–372.

1953, Effects of temperature on spontaneous and induced mutations in *Escherichia coli*. Proc. Nat. Acad. Sci. 39: 427–433.

Zinder, N. D., and J. Lederberg, 1952, Genetic exchange in Salmonella. Jour. Bact. 64: 679–699.

USE AND MISUSE OF THE BIOTIC PROVINCE CONCEPT

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It has been forty years since Vestal (1914, p. 432) proposed the term "biotic province" in the pages of this journal, and the principles underlying that term have undergone considerable refinement since then. Dice (1943) crystallized the concept and applied it to North America, which he divided into twenty-nine provinces. Each province, according to Dice (1943, p. 3), "covers a considerable and continuous geographic area and is characterized by the occurrence of one or more important ecologic associations that differ, at least in proportional area covered, from the associations of adjacent provinces. In general, biotic provinces are also characterized by peculiarities of vegetation type, ecological climax, flora, fauna, climate, physiography, and soil." This implies, and partly states, that there is a continuity within and a discontinuity between biotic provinces. Difficulty is often experienced in locating the boundary between adjacent provinces, however, and frequently they merge gradually into each other (Dice, loc. cit., p. 4).

Vestal had two criteria for using the term "biotic province." One of these was the similarity of geographic range among ecologically similar animals; the second was closeness of correspondence of distribution of particular animals with that of vegetation provinces. His unit was to be based upon the biological *tout ensemble*. His awareness of one of the major stumbling blocks in the use of the concept is embodied in his statement (1914, p. 444) that "the more restricted in area, or uniform in biological conditions, this region is, the greater uniformity of the collection of species." Dice (1943, p. 6) felt that "the limits of geographic range of species and races of plants and animals are not fully satisfactory criteria for determining the boundaries of biotic provinces...." Dice leaned heavily on ecological principles for his delimitation for he stated (1943, p. 5) "the classification of biotic provinces should properly be based upon the distinctness and distribution of the various ecologic associations," and further (1943, p. 4) "each biotic province is characterized usually by a single climax association...." The difficulty in meeting these requirements in their entirety is fairly obvious, however, and many zoogeographers have found it convenient to work with smaller divisions of the biota in their analysis of provincial divisions. Thus, Burt (1938, p. 11) discussed "Faunal relationships and biotic provinces in Sonora, based on recent mammals." Blair (1950) correlated the distribution of terrestrial vertebrates with vegetation types in his work on the biotic provinces of Texas. Mello-Leitao (1942, p. 132) mapped the provinces of South America, as defined by the distribution of the scorpion fauna. That this practice has proven fruitful,

and permits extrapolation of the data to fit other animal groups, is a testimony to the care with which these authors have worked. It will be pointed out below, however, that certain strictures must be placed upon both the technique and the extrapolation.

Smith (1949) has pointed out that the practice of delimiting biotic provinces has been "...hindered by an almost universal lack of agreement upon the magnitude of subdivision (based upon percentage of forms influenced by it) implied by the biotic province." Although inadequate data from sizable areas is a partial cause of this disagreement, Smith felt that complete data would almost certainly not suffice to remedy it, due to lack of duplication of any two cases. As a solution to this dilemma, he suggested that "a ...reasonable method of standardization would be the arbitrary establishment of limits of faunistic distinctiveness for various subdivisions—including biotic provinces—of major regions." To achieve this standardization, Smith suggested the selection of a sample area for study (which he thought should be California and adjacent areas) and the formulation of index figures, by percentage distinctiveness, for each of three groups of terrestrial vertebrates (reptiles and amphibians, mammals, and birds). The use of specified groups of animals is a recognition of the unlikelihood of future students being able to analyze an entire fauna at one time, and would be a continuation of the practices already mentioned above.

Smith did not discuss methods for calculating the percentage of distinctiveness of a fauna. I can visualize at least two factors on which such a figure might be based. First, the number of endemic taxons in any particular geographic unit could be compared with the taxons shared with neighboring geographic units. Second, the number of taxons which find their range limits either at the boundaries of or within the geographic unit could be compared with the number whose ranges do not appear to be coincident with those of the unit. Thus, taxons which pass a faunal break at one end of a geographic unit could still be useful in the definition of a similar break at the opposite end, if their ranges terminated there. The distinctiveness of a faunal unit should be expressed as a percentage based upon these two factors, with perhaps a weighting factor included to account for varying levels of taxonomic differentiation. This weighting would emphasize the greater significance of a species limit as compared to a subspecies limit.

In order to test these possibilities, and to investigate the likelihood of their proving fruitful, I have subjected the reptile and amphibian fauna of one of the biotic provinces of Mexico to a close scrutiny from the point of view of the fauna's substantiation of the validity of the province's boundaries. While I agree with Smith that the region that could be most profitably analyzed in this fashion would be one as intensively collected as California, I am forced to study that area with which I am most acquainted, the southwestern coast of Mexico.

The Mexican biotic province under consideration has been called the Lower Balsan Province (Smith, 1939); the Nayarit-Guerrero Province (Goldman and Moore, 1946); or the Acapulcan Province (Smith, 1949). I shall

continue the practice adopted in several of my earlier papers on the area, and call it the Nayarit-Guerrero Province. As defined by Goldman and Moore (1946, p. 355) this Province "occupies the coastal region within the Arid Tropical Zone from southern Sinaloa, south through Nayarit, western

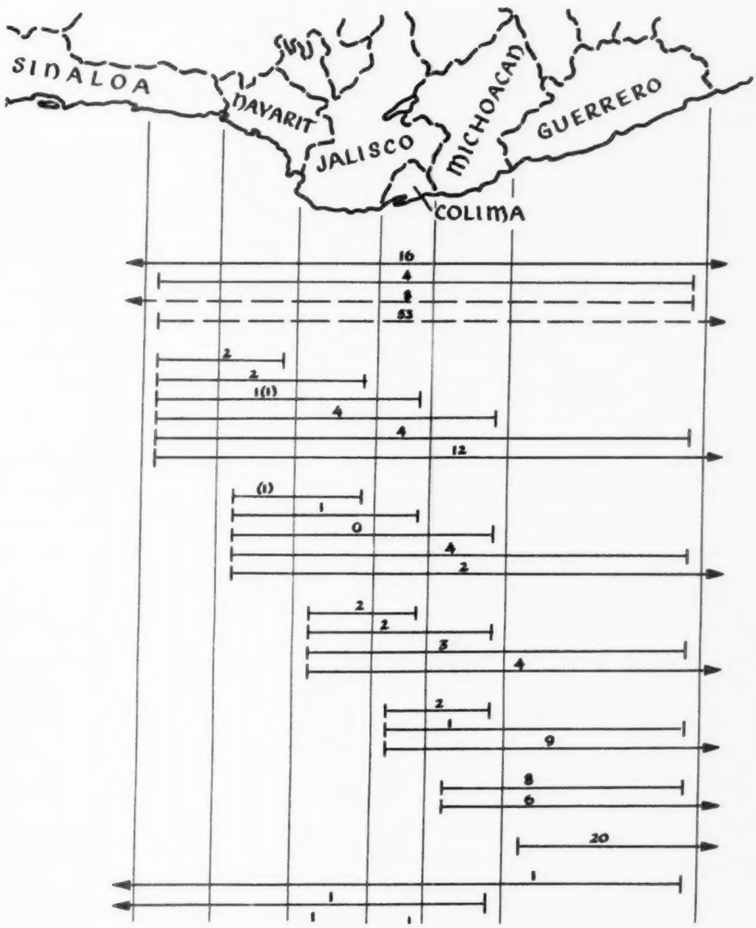

FIGURE 1. The Nayarit-Guerrero Biotic Province. Each line represents a distributional pattern, and the number above the line represents the number of taxons that exhibit that pattern. Lines with arrows represent forms extralimital in the direction indicated. The broken lines represent combined totals of all species extralimital in one direction only, and these figures are included in the breakdown state by state that follows. Only those species known from two or more states are included, and parenthetical figures refer to species for which the data are not entirely reliable.

Jalisco, Colima, southwestern Michoacán, and southern Guerrero." The region is shown on the accompanying map (fig. 1). It will be noted that these Mexican states are arranged like a string of boxcars, and thereby facilitate an analysis state by state. Since the reptiles and amphibians of Mexico have been listed in detail by Smith and Taylor (1945, 1948, 1950) and have been tabulated state by state therein, the analysis of the herpetofauna has been greatly simplified. I have had field experience in each of the states included in the province, with the single exception of Sinaloa. A paper dealing with some of the material collected by myself and others in the area under consideration is available (Peters, 1953), and should be consulted for detailed data not included in this study.

ANALYSIS OF THE HERPETOFAUNA

A total of 150 species and subspecies of reptiles and amphibians have been collected and recorded from the province. Of this total 16 are extralimital in both directions, ranging north to Sonora or farther and to Oaxaca or farther on the south. Eight forms are extralimital to the north but have the southern limit of their range within the province. Fifty-two forms are extralimital to the south. Thus a total of 76 forms are extralimital to some degree, and slightly more than 50 per cent of the total fauna becomes contradictory evidence as far as the establishment of the boundaries is concerned. The remaining 74 forms, however, would appear to be strong confirmatory evidence, for the entire range of each is contained within the province. Further analysis of this group of forms shows that this is not an entirely reliable method of approach. Thirty-two of the forms are known from only one or two specimens, and these few specimens come from the type locality of the species, in most cases. Most of them would remain endemics if each Mexican state was called a biotic province, and would be just as good evidence of the validity of such a unit. Such rare and extremely restricted forms are no more valuable in discerning biotic relationships than those forms which have wide tolerance and extensive ranges.

All of the remaining 42 forms, however, are available for additional study, and we can also add the forms extralimital in one direction but contained in the other. Of all of these, only four are truly representative of the province, in that their ranges coincide with that given for it. The remaining forms, while all contained within or limited by the province, display many other units of range limits, some of which contain more coincident species than does the recognized province (fig. 1).

The map shows the step-wise fashion in which the forms are limited. Thus, of the nine forms with their southern limit in Michoacán, two are known to extend only to Colima, two others range into Jalisco, four reach Sinaloa, and one is extralimital at the northern boundary of the province. When those forms that are extralimital to the south are broken down as to their northern limit the sequential loss is again obvious. Twenty stop in Guerrero, five more in Michoacán, nine in Colima, four in Jalisco, two in Nayarit and twelve in Sinaloa.

Since the portions of the states within the province are of approximately equal size (with the exception of Colima), we can conveniently break the ranges down to a matter of presence or absence and tabulate the number of species or subspecies with a range limit within each state (table 1). Although the state of Sinaloa is not entirely within the province, all taxons with their northern limit in that state have been included in arriving at the given figure, and 24 forms fit that category. All of these thereby contribute to the establishment of the northern limit of the province. If, however, the tiny state of Colima is combined with either Jalisco or Michoacán (an act justified by its size), the sum of the limited species reaches the order of magnitude given for Sinaloa. This fact is particularly startling when it is remembered that the northern end of this province is generally considered to coincide with the boundary of the Neotropical Realm. One might have expected far more species to be limited by this boundary than actually are. It can hardly be questioned that a break of some sort takes place at the southern limit of the province, for 20 forms find their northern limit in Guerrero, and 21 find a southern limit there. Again, the order of magnitude is directly comparable with that of Colima combined with Jalisco or Michoacán.

TABLE 1

A COMPARISON OF THE NUMBER OF TAXONS LIMITED BY EACH STATE IN THE NAYARIT-GUERRERO PROVINCE. THE STATES ARE ARRANGED FROM NORTH TO SOUTH IN DESCENDING ORDER. PARENTHETICAL FIGURES REFER TO FORMS FOR WHICH THE KNOWN DATA ARE NOT ENTIRELY RELIABLE.

	Species and Subspecies		Genera	
	Northern limit	Southern limit	Northern limit	Southern limit
Sinaloa	25(1)		4	
Nayarit	7(1)	4	5	(1)
Jalisco	11	5(1)	5	2
Colima	12	5(1)	7(1)	1
Michoacán	14	9	4(1)	0
Guerrero	20	21	9	6

Comparison on the generic level is quite fruitful. The 150 species and subspecies found in the province represent 79 genera, of which 34 find their northern limit therein, and 9 find their southern limit. Guerrero again has the largest total, but combination of Colima with Jalisco or Michoacán again raises that number to an equivalent of Guerrero. All of the genera limited by Sinaloa are tropical in distribution, and verify its status as the end of the tropics, to some extent. Colima itself limits almost twice as many genera on the north as does Sinaloa, however, and every one of them is a tropical genus. In addition, many tropical genera are known to range well into Sonora and southwestern United States.

DISCUSSION

The data presented indicate that faunal breaks as great as that which takes place in Sinaloa can be found elsewhere within the Nayarit-Guerrero Province. This might mean that the Sinaloa boundary should be de-emphasized, or if we were to base our decision entirely upon percentage figures, that the other breaks should be recognized as provincial limits. It should be recognized that a certain amount of fallacious reasoning leads to the first alternative, since it assumes that the Neotropical realm can be delimited by a sharp line. As pointed out above, Dice recognized the frequency of a gradual merging of provinces. This applies equally well, if not with greater force, to the zoogeographic realms of the world, and it is likely that several biotic provinces would be included within the broad overlap between major realms. The second alternative does not take into account the fact that range limits, to our knowledge, do not coincide within the state any more than within the entire province, and that equally smaller units might be set up within the state by judicious selection of areas. Obviously, neither of the two alternatives suggested above provide satisfactory answers to the dilemma.

The difficulty appears to arise from a fact of consequence not only in this restricted area under discussion above, but also in all studies of a fauna or biota. It seems to me that the study of biotic provinces includes clinal variation. Huxley (1943, p. 206) points out that "since gradients in environmental factors are a widespread feature of the environmental mould, we should expect organisms to show corresponding adaptive gradients in their characters." He applied the word "cline" to these gradients in characters. A biota as well as each of its component species is capable of responding to its environment in a step-wise fashion, and a gradient in the environmental factors might result in a gradient within the province. The cline would be expressed in a gradual dropping out of species in response to gradual changes, and could be expressed on a percentage basis. Thus, the Nayarit-Guerrero province may well represent a clinal response just as some of the species contained in it are known to show clinal variation. One encounters the same difficulties in trying to give the province a name and delimitation as does a worker who finds it necessary to circumscribe the variations of a clinal species, since the variations do not exist as a unit, and therefore cannot easily be described as such.

If this be true, it seems unlikely that any plan to describe biotic provinces on a statistical or empirical basis will be uniformly successful. It will certainly encounter a major obstacle when applied to provinces such as the one under discussion. Clinal variation in a species seems to require a continuum of physiographic factors accompanied by gradual change in climatic factors of the environment. For example, clinal variation has been observed in several different animal groups ranging across the Interior Plains of the United States, showing both north-south and east-west trends. The close relationship between the physiography and the biota of an area

is clearly seen if Dice's map of the biotic provinces of North America (1943, opposite p. 4) is compared with a physiographic map of the United States, and this has long since been pointed out by Dunn (1922). Obviously, distinct breaks in the landscape are accompanied by distinct breaks in the biota. Isolation of one set of factors such as physiographic from another such as climatic is physically impossible, and their interrelationships will continue to complicate the picture in all cases, of course, but it seems plain that the situation is not always one of clear-cut continuity and discontinuity. A province such as the Nayarit-Guerrero, lying as it does on a north-south axis, and possessing a fairly uniform physiography, might be expected to display a step-wise loss of forms. In addition, any province lying near a major change in biogeographic realms would be caught in the overlap and the gradual loss might be anticipated. The biotic province has considerable value to biogeographers as a concept, of course, and will continue to be useful in the analysis of distributional problems. The same touchstone of caution that is currently applied to any evaluation of variation within a species can and should be utilized by biogeographers, however, and an awareness of the possible pitfall of clinal change be omnipresent in their minds.

This caution can be most successfully observed by systematists in their analysis of various taxons. The definition of a poorly characterized species or subspecies as new on the basis of its occurrence in a different biotic province than the typical form is to be avoided. The delimitation of the range of a known species on the basis of the limits of the province in which it is known to occur should be done only on the basis of accurate collecting data, not on probability. The latter practice is particularly insidious in that the province is originally defined on the basis of the combined ranges of its inhabitant species, and a species range defined on the basis of the provincial limits may later turn up as an additional verification of the accuracy of those limits.

SUMMARY

The biotic province concept shows strong similarities to the concepts involved in the analysis of infraspecific variation by systematists, both in its definition and application. This similarity is used as a basis for the extrapolation of ideas concerning infraspecific variation of a clinal nature into the study of biotic provinces. The Nayarit-Guerrero Province of Mexico can be shown to exhibit many of the responses typical of clinal variation (as defined in this paper). This fact is an obstacle to standardizing the definition of biotic provinces on the basis of percentage distinctiveness of its fauna or flora.

The concept of the biotic province is abused when it is employed as evidence of the validity of a new species or subspecies. It should not be used as the basis for range definition of any form unless verified by accurate and adequate collecting information. Care should be exercised at

all times that the interdigitation of species range and province definition does not lead to circuitous reasoning as to the independent validity of each.

It is unlikely that the establishment of regulations concerning the percentage distinctiveness of a biota as a means of definition of biotic provinces will prove successful, due to the step-wise or clinal loss of taxons from the fauna.

LITERATURE CITED

Blair, W. F., 1950, The biotic provinces of Texas. Texas J. Sci., Vol. 2: 93–117.

Burt, W. H., 1938, Faunal relationships and geographic distribution of mammals in Sonora, Mexico. Misc. Publ. Mus. Zool. Univ. Mich., No. 39: 1–77.

Dice, L. R., 1943, The biotic provinces of North America. Ann Arbor, Univ. Mich. Press: vii + 78.

Dunn, E. R., 1922, A suggestion to zoogeographers. Science, Vol. 56: 336–338.

Goldman, E. A., and R. T. Moore, 1946, The biotic provinces of Mexico. J. Mammal., Vol. 26: 347–360.

Huxley, J., 1943, Evolution, the modern synthesis. London, Harper and Brothers: 1–645.

Mello-Leitao, C., 1942, Los Alacranes y la Zoogeografia de Sudamerica. Rev. Argentina Zoog., Vol. 2: 125–131, 2 maps.

Peters, J. A., 1954, The amphibians and reptiles of the coast and coastal sierra of Michoacán, Mexico. Occ. Pap. Mus. Zool. Univ. Mich., no. 554: 1–37.

Smith, H. M., 1939, The Mexican and Central American lizards of the genus *Sceloporus*. Zool. Ser., Field Nat. Hist., Publ. 445: 1–397.

1949, Herpetogeny in Mexico and Guatemala. Annals Assoc. Amer. Geog., Vol. 39: 219–238.

Smith, H. M., and E. H. Taylor, 1945, An annotated checklist and key to the snakes of Mexico. United States Nat. Mus., Bull. 187: 1–239.

1948, An annotated checklist and key to the amphibia of Mexico. *Ibid.*, Bull. 194: 1–118.

1950, An annotated checklist and key to the reptiles of Mexico exclusive of the snakes. *Ibid.*, Bull. 199: 1–253.

Vestal, A. G., 1914, Internal relations of terrestrial associations. Amer. Nat., Vol. 48: 413–445.

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BUFFERED GENOTYPES AND IMPROVEMENT IN EGG PRODUCTION

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A number of investigators in population genetics have recently turned their attention to the genetic basis and evolutionary implications of physiological homeostasis. Genetic variation in the ability of individuals to proceed to the realization of adapted adult form and function with some degree of independence from uncontrolled fluctuations of their immediate milieu is an important factor in determining Darwinian fitness. Hence, it has been argued that natural selection has favored genotypes which are buffered, that is to say, genetic combinations which enable the organism to withstand the vicissitudes imposed on it by the vagaries of its environment. Various aspects of such self-regulating properties in development and reproduction have been referred to as plasticity (Salisbury, 1940), canalized development (Waddington, 1940), existential adaptation (Goldschmidt, 1948), stability (Mather, 1953), phenotypic flexibility (Thoday, 1953) and otherwise. Perhaps, the simplest way of describing individual capacity to maintain steady states, at least in the context of the present discussion, is by the term buffering ability (Waddington, 1940), while genotypes endowing the organism with homeostatic properties may be referred to as buffered genotypes.

There are many aspects of the genetics of buffered behavior which are of significance in evolutionary theory. They include stabilizing (Schmalhausen, 1949) and normalizing (Waddington, 1953a) selection, also known by a variety of other designations, genetic assimilation (Waddington, 1953b), the rôle of heterozygosity in population structure (Dobzhansky and Wallace, 1953; Lerner 1954a and b), and others. There is, however, also at least one phase of the current experimentation and speculation in this field which has a strong bearing on the problems of applied breeding, and which deserves comment. This aspect can be illustrated particularly with respect to egg production in poultry.

Probably the earliest reference to the subject in relation to poultry breeding was made by Munro (1936), who first suggested and later, with his collaborators (Munro, Kosin and Macartney, 1943), more explicitly described in this journal the hypothesis that variability in egg production is conditioned in part by genes "protecting" birds from environmental mishaps. Munro's formulation, however, differs in a rather important way from that presented here. He considered that genetic variation in homeostatic properties not only could not be utilized in a program of selective improvement but actually impeded it. His argument was that protective or buffering "secondary" genes interact with the unique environment of every individual so as to obscure the phenotypic expression for the "primary" genes re-

sponsible for genetic variability in the egg record, and thus interfere with the accuracy of selection. In other words, Munro's notions of buffering behavior visualized a strong specificity of "protective" genes (cf. Williams', 1950, concept of genetotrophic disease). In this view, such genes exercise their effects only under exposure to a specific environmental stimulus, in the absence of which they have no selective value. Thus an animal may be chosen as one of the parents of the next generation on the strength of its ability to cope with a non-recurrent environmental situation, peculiar to that particular animal. Since its offspring will not encounter the identical situation, selection pressure would have been applied in an inappropriate direction, and no gains in performance would be obtained.

There is of course considerable merit in the hypothesis presented by Munro. It is clear, for instance, that selection which has as its result an increase in the frequency of genes conveying resistance to some disease is of no value if the subsequent generations do not encounter this particular disease. In a broader way the same applies to climatic conditions existing at the particular time when performance on which selection is based is measured, but which may not be repeated when the progeny of the selected birds is tested. Inter-year gene-environment interactions of this type will attenuate selection progress. However, the intra-year interactions of the individual genotype with its own environment does not necessarily present such a critical problem, especially under family selection schemes. Furthermore, buffered behavior, as here defined, while possibly mediated by homeostatic mechanisms specific to each situation, is a reflection of the total capacity of the genotype for self-regulation. The distinction between primary and secondary genes seems to be gratuitous, Indeed, although specific major gene action may play a part in the performance of poultry flocks, it is more likely that the important source of genetic variability lies in polygenes with interchangeable rather than bottleneck effects (Lerner, 1950). These should be amenable to selection pressure, even though their dominance and epistatic interactions may call for complicated breeding systems if full advantage of the available genetic variance is to be taken.

There is some evidence that selection for high egg production has been, at least to a degree, based on selection for better buffering. None of it is conclusive in itself. Most of it is vague and indirect. Yet the total picture is such as to suggest that a fruitful field of investigation lies in the study of buffered behavior which may prove to have significant repercussions in future breeding practice.

Before noting the various observations bearing on this suggestion, an important premise has to be mentioned. It deals with the hypothesis that buffered behavior of cross-fertilized organisms rests on a certain obligate level of heterozygosity. The rationale underlying this assumption and the evidence on which it is based are reviewed elsewhere (Lerner, 1954a and b). Suffice it to state here that phenotypic variability of genetically homogeneous populations is an index of buffering capacity, since it measures the degree of departure of individual phenotypes from the average or norm of

the group considered. There are many data ranging from specially designed experiments (such as those of Dobzhansky and Wallace, 1953, on Drosophila) to the mass observations on hybrid corn as noted by Richey (1950), which show that in cross-fertilized species characters of adaptive value tend to vary less among heterozygous individuals than among homozygotes of a given kind. Thus, the superior egg production of crosses between inbred lines of poultry (e.g. in the report of Stephenson, Wyatt and Nordskog, 1953) may be interpreted as being due to the fact that heterozygotes are less liable to be adversely influenced by environmental fluctuations than homozygotes.

The ancestors of the domestic fowl had a limited period of egg production in the spring and summer (Pearl and Surface, 1911). Probably the main changes in this respect achieved under domestication and selection lie in the suppression of most, if not all, of the broody periods within the original breeding season, and, more significantly, in the extension of the laying year to include the fall and winter. While the expression of broodiness is conditioned in part by environmental stimuli (Burrows and Byerly, 1938), its dependence on complementary gene action (Goodale, Sanborn and White, 1920) leads to a high level of incidence in many hybrids (Knox and Olsen, 1938). There is, thus, some evidence that an increase in broodiness in crosses of improved strains and breeds is an aspect of heterotic behavior, particularly because a positive association between broodiness and viability has been noted (Byerly and Knox, 1942). One may then argue that environmental shocks suppress broodiness, and that possession of this economically undesirable, but biologically necessary, character is an example of high buffering capacity.

More significantly, the extension of the laying year suggests a greater independence of the improved populations from their environment. The high producing bird hatched in the late winter or early spring begins to lay earlier than its progenitor (a property which in the wild may not have been useful), in spite of the fact that its pituitary is not exposed to the type of stimulation by light that the unimproved bird apparently needs (cf. the situation in "semi-improved" turkeys as reported by Asmundson, Lorenz and Moses, 1946). Similarly such a bird continues to lay late in the fall, though the length of day still exercises an effect on the date of cessation of production (Lerner and Taylor, 1941).

Not only is the variation in maturity and persistency affected by external conditions, but even more so may be the tendency to pause. Thus when Greenwood (1954) maintained a group of birds protected from environmental fluctuations, he observed a startling improvement in this respect over control birds kept in ordinary pens. The egg production performance of birds kept in individual cages points to the same fact from a different angle. Probably the most significant difference between the records of birds in cages and those on the floor lies in the lowered variability of egg records (Bird, 1937, confirmed by unpublished observations in our laboratory). The reduction in the variance is produced not only by elimination of the left tail

of the frequency distribution (which may be expected because of the unsatisfactory performance on the floor of birds low in the social order) but also by limitation of the proportion of birds with high records.

This phenomenon may be explained on the reasonable assumption that birds on the floor can to some extent control their own environment, for example, by seeking refuge from heat in a shady part of the pen, while the birds in cages are completely at the mercy of their immediate milieu. These birds must depend on their homeostatic mechanisms to protect them from impingement of environmental changes. Thus, it is reported from the field that certain breed crosses excel purebreds in production under cage management more than they do on the floor. The high self-regulatory capacity of hybrids may well be the explanation of this alleged fact.

That there are genetically determined differences in ability to withstand stress can be probably deduced on *a priori* grounds. There is, however, more definite information on this point, which is being obtained currently. For instance, in a series of unpublished experiments by Lerner and Gunns it has been established that inbred lines, crosses between them, and openbred populations differ in their ability to recover from temperature shocks during incubation. Under the particular type of treatment used, the hatchability (as per cent of that of untreated controls) of a strain selected for egg production was 55 per cent as compared to 35 per cent for inbred lines and 75 per cent for crosses between them. Data on the correlation between homeostatic capacity with respect to such shocks and egg production within the production-bred line are being collected now.

One does not need to assume that all genetic variation in egg production depends on buffering capacity. Nevertheless, the positive genetic correlations between production and viability (Dempster, Lerner and Lowry, 1952), correlated responses in egg production under selection for resistance and susceptibility to lymphomatosis (Hutt and Cole, 1947), positive genetic correlations between incidences of death from different causes (reasonably low in the report of Robertson and Lerner, 1949, and high in that of Lush, Lamoreux and Hazel, 1948), the association between low rate and pausing incidence (Lerner and Taylor, 1947)—all argue for the significance of general vigor, or buffering ability, as a determinant of performance in egg production.

Further support for this hypothesis can be found in Gowe's (1952) observation on the lack of significant interaction between genotype and environment at a series of Canadian Experiment Stations. However, other data from the same source (Gowe, Johnson and Wakely, 1953) indicate that heritability estimates of egg production are affected by environmental differences, which may mean that the primary characters measured at different locations may not be identical, though they are expressed phenotypically on the same scale. It may be parenthetically mentioned here that the proportion of genetic variation that is due to general factors operating in all environments to that produced by factors specific for a given environment could not be expected to remain constant. It may shift up or down depending on the ge-

netic biography of a given population, since different types of selection could change gene frequencies determining the denominator and the numerator of this ratio independently of each other.

Be this as it may, the various points considered here suggest that it may be worthwhile to investigate the genetics of stress response in chickens with a view of determining whether or not correlated responses in egg production may be obtained by selection for buffering capacity. If this property is related to heterozygosity, systems of breeding designed to utilize heterosis would assume an even greater significance in practice than they do now. Whether crossing of inbred lines, crossing of strains or breeds, reciprocal recurrent selection, selection on basis of a constant tester, or schemes similar to the balanced population technique, such as has been proposed by Brieger (1950) for corn, will eventually replace closed flock selection is, of course, a different problem, which bears only indirectly on the matters discussed here.

SUMMARY

It is suggested that genetic variation in egg production depends to a degree on buffering capacity. Observations in support of this hypothesis are briefly reviewed.

LITERATURE CITED

Asmundson, V. S., F. W. Lorenz and B. D. Moses, 1946, Influence of light intensity on ovulation in turkeys. Poultry Sci. 25: 346-354.

Bird, S., 1937, Fecundity and reproductive ability in closely confined fowl. Sci. Agriculture 17: 359-375.

Brieger, F. G., 1950, The genetic basis of heterosis in maize. Genetics 35: 420-445.

Burrows, W. H., and T. C. Byerly, 1938, The effect of certain groups of environmental factors upon the expression of broodiness. Poultry Sci. 17: 324-330.

Byerly, T. C., and C. W. Knox, 1942, Broodiness and viability. Poultry Sci. 21: 370-373.

Dempster, E. R., I. M. Lerner and D. C. Lowry, 1952, Continuous selection for egg production in poultry. Genetics 37: 693-708.

Dobzhansky, Th., and B. Wallace, 1953, The genetics of homeostasis in Drosophila. Proc. Nat. Acad. Sci. 39: 162-171.

Goldschmidt, R. B., 1948, Ecotype, ecospecies, and macroevolution. Experientia 4: 465-472.

Goodale, H. D., R. Sanborn and D. White, 1920, Broodiness in the domestic fowl. Mass. Agr. Exp. Sta. Bull. 199: 93-116.

Gowe, R. S., 1952, Recent information on the relative effect of environment and heredity on egg production. Poultry Sci. 31: 918-919.

Gowe, R. S., A. S. Johnson and W. J. Wakely, 1953, The effect of location on the heritability of egg production of two S. C. White Leghorn strains. Poultry Sci. 32: 901.

Greenwood, A. W., 1954, Improving on nature. Farmers' Weekly 40: 70-71.

Hutt, F. B., and R. K. Cole, 1947, Genetic control of lymphomatosis in the fowl. Science 106: 379-384.

Knox, C. W., and M. W. Olsen, 1938, A test of crossbred chickens, Single Comb White Leghorns and Rhode Island Reds. Poultry Sci. 17: 93-99.

Lerner, I. M., 1950, Population genetics and animal improvement. Cambridge Univ. Press, England.

1954a, Genetic homeostasis. Oliver and Boyd, Edinburgh.

1954b, The genotype in Mendelian populations. Proc. 9th Int. Cong. Genetics, Bellagio (in press).
Lerner, I. M., and L. W. Taylor, 1941, Factors affecting the duration of the first annual rest. Poultry Sci. 20: 490–495.
1947, Further observations on winter pause in Single Comb White Leghorn pullets. Poultry Sci. 26: 198–205.
Lush, J. L., W. F. Lamoreux and L. N. Hazel, 1948, The heritability of resistance to death in the fowl. Poultry Sci. 27: 375–388.
Mather, K., 1953, Genetic control of stability in development. Heredity 7: 297–336.
Munro, S. S., 1936, The inheritance of egg production in the domestic fowl. Sci. Agriculture 16: 591–607.
Munro, S. S., I. L. Kosin and E. L. Macartney, 1943, Quantitative genic-hormone interactions in the fowl. Amer. Nat. 77: 256–273.
Pearl, R., and F. M. Surface, 1911, A biometrical study of egg production in the domestic fowl. U.S.D.A. Bur. Animal Ind. Bull. 110: 81–170.
Richey, F. D., 1950, Corn breeding. Adv. Genetics 3: 159–192.
Robertson, A., and I. M. Lerner, 1949, The heritability of all-or-none traits: Viability of poultry. Genetics 34: 395–411.
Salisbury, E. J., 1940, Ecological aspects of plant taxonomy. New Systematics 329–340.
Schmalhausen, I. I., 1949, Factors of evolution. Blakiston. Philadelphia.
Stephenson, A. B., A. J. Wyatt and A. W. Nordskog, 1953, Influence of inbreeding on egg production in the domestic fowl. Poultry Sci. 32: 510–517.
Thoday, J. M., 1953, Components of fitness. Symp. Soc. Exp. Biol. 7: 96–113.
Waddington, C. H., 1940, Organisers and genes. Cambridge Univ. Press, England.
1953a, Epigenetics and evolution. Symp. Soc. Exp. Biol. 7: 186–199.
1953b, Genetic assimilation of an acquired character. Evolution 7: 118–126.
Williams, R. J., 1953, Concept of genetotrophic disease. Nutrition Rev. 8: 257–260.

RECESSIVE AND SPORADIC RUMPLESSNESS OF FOWL: EFFECTS ON PENETRANCE AND EXPRESSIVITY[1]

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Complete or partial absence of the tail vertebrae and of the associated soft tissues occurs in fowl as a consequence of at least two independent gene substitutions. One of these variants is the well-known rumpless fowl of fanciers in which lack of the tail is transmitted as a dominant trait. The second type of rumplessness is represented by a recessive mutation (Landauer 1945). In the absence of modifying genes, the two mutant forms show a similar morphology of the pelvic skeleton, though the epigenetic processes of origin are very different ones (Zwilling 1942, 1945); recessive rumplessness, however, is frequently associated with additional skeletal abnormalities, such as supernumerary ribs or a lordotic and kyphotic spine.

The expression of both rumpless mutations is affected by multiple, recessive modifying genes which appear to be an ubiquitous part of the normal genotype of all breeds and stocks of fowl that have been tested (Dunn and Landauer 1934, 1936; Landauer, 1945). In the presence of such modifying genes the intermediate phenotypic expressions of the two mutants can usually be told apart by minor morphological peculiarities. In both instances, the accumulation of plus modifiers tends to lead, by imperceptible gradations, to forms that are indistinguishable from the normal phenotype. It was presumably in such a completely submerged condition that the genes for recessive rumplessness had been secluded in our White Leghorn stock until they found expression in the F_2-generations of outcrosses to Silver Spangled Hamburg and Creeper fowl.

In crosses involving the gene for dominant rumplessness segregation is quite normal and full expression of the mutation can readily be maintained by selective exclusion of modifiers. This is most easily accomplished in backcrosses of rumpless hens to normal cocks since (and this holds for both mutant stocks) *inter se* matings of rumpless fowl or the use of rumpless males results in more or less complete loss of fertility on account of mechanical difficulties in copulation. In our stock of recessive rumpless fowl penetrance of the mutation was low and animals with complete lack of the tail vertebrae were relatively rare (low expressivity). The possibility of selecting toward more extreme phenotypic expression was in this case limited by the necessity of maintaining a sufficient degree of fertility. We decided, therefore, to test if penetrance and expressivity of recessive rumplessness could be enhanced by changes in the residual heredity.

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The crosses that we made for this purpose were based on the occurrence of a third type of tail defect which we may designate as sporadic rumplessness. It is well known that in most (perhaps all) breeds of fowl (completely) rumpless embryos and chicks are found on occasion, the great majority of which die during the last days of incubation. The incidence of this malformation varies between breeds and stocks. The strain of White Leghorn fowl from which our recessive rumpless stock had been isolated produced 1.60 ± 0.40 per cent of rumpless embryos and chicks among the survivors of the thirteenth day of development (N = 1000). Among the breeds at our disposal, the lowest incidence of sporadic rumplessness was found in Red Jungle fowl with 0.62 ± 0.25 per cent (N = 1455), and the highest frequency occurred in Silver Gray Dorking fowl with 5.75 ± 0.91 per cent (N = 2434). Incidence in the Dorking stock exceeded that among the Leghorns by 4.15 ± 0.99 per cent, clearly a significant difference. The frequency of sporadic rumplessness in our stock of Jungle fowl was 0.98 ± 0.47 per cent below that found in our Leghorns, a difference which is probably significant (standard errors), and the difference between Dorking and Jungle fowl was, of course, *a fortiori* highly significant (5.13 ± 0.94 per cent). The ease with which rumplessness can be produced experimentally, e.g. by the injection of insulin into eggs prior to or soon after the beginning of incubation, also differs significantly between Dorking and Jungle fowl. The incidence of rumplessness after injecting 2 units of insulin at 0 hours is (among the survivors of the thirteenth day of incubation) in Dorking embryos about three times that found in embryos of Jungle fowl.

It is clear that these breeds differ in their "disposition" to produce sporadic rumplessness, just as the same was found for taillessness in several stocks of rats (Dunn, Gluecksohn-Schoenheimer, Curtis and Dunning, 1942). Attempts at a further genetic analysis and of raising incidence by selection have so far been fruitless, but new experiments are now under way. It must be emphasized, however, that—at least in so far as our stock of Dorking fowl is concerned—intra-breed heterogeneity for the production of sporadic rumplessness can be demonstrated. An analysis of the data for five pen matings of Dorking fowl, with progenies between 193 and 525 individuals, yielded for the combined probabilities a χ^2 of 28.47 with 10 degrees of freedom and a probability of $<.005$.

On the basis of the foregoing information, we decided to cross our recessive rumpless stock to Jungle and Dorking fowl and to compare penetrance and expressivity of recessive rumplessness among the descendants of these crosses and with the corresponding data for the foundation stock. The results are summarized in table 1. The last segregation data given in our earlier publication (Landauer, 1945) had been for 1940 and 1941. Between that time and 1947, the year when the present experiment was started, the recessive rumpless stock had not changed significantly in either the segregation ratio of rumpless phenotypes or in the relative incidence of completely rumpless animals among all individuals with tail defects.

The F_3-progenies from the crosses of recessive rumpless fowl to Dorkings and Jungles, respectively, diverged widely from each other. In the F_3 from the Dorking cross the incidence of all types of tail abnormalities was 40.7 per cent, whereas for the F_3 from the Jungle cross the corresponding figure was 7.8 per cent. The difference is highly significant ($\chi^2 = 85.17$, df 1, $P < .001$). Again, in the Dorking-recessive rumpless F_3-generation 53.4 per cent of the progeny were completely rumpless, but only 18.8 per cent of the F_3-descendants from the cross to Jungle fowl showed entire lack of tail vertebrae. This difference, after correction for the smallness of the sample, is also significant ($\chi^2 = 6.158$, df 1, $P < .02$). It is clear, therefore, that in these two genotypic environments penetrance and expressivity of the recessive rumpless condition have diverged in directions corresponding to the differences in incidence of sporadic rumplessness found in the original stocks. It should be pointed out in this connection that all those descendants of the Dorking and Jungle crosses with tail defects which were tested in later matings proved to carry the recessive rumpless mutation.

TABLE 1

DETAILS OF SEGREGATION FOR RECESSIVE RUMPLESSNESS IN THE ORIGINAL WHITE LEGHORN-DERIVED STOCK AND IN THE F_3 GENERATIONS OF CROSSES TO SILVER GRAY DORKING AND RED JUNGLE FOWL

Parents (intermediate rumpless)	Size of progeny	Normal rump	Intermediate rumpless	Completely rumpless	Incidence of all types of rump defects (penetrance) %	Incidence of complete rumplessness among all rump defects (expressivity) %
Recessive rumpless (White Leghorn) 1940 and 1941	499	319	152	28	36.1	15.5
Recessive rumpless (White Leghorn) 1947	234	157	61	16	32.9	20.8
F_3 (Dorking × recessive rumpless)	1686	1000	320	366	40.7	53.4
F_3 (Jungle × recessive rumpless)	206	190	13	3	7.8	18.8

If the data of the F_3-progenies from the Dorking and Jungle crosses are compared with the (1947) foundation stock of recessive rumpless fowl, the differences are, obviously, less pronounced. Yet, in the instance of the descendants of the Dorking cross the penetrance of rumplessness is definitely increased ($\chi^2 = 5.458$, $P < .02$) and the frequency of complete rumplessness (expressivity) is greatly raised ($\chi^2 = 29.50$, $P < .001$). A comparison of the F_3-progeny from the Jungle fowl cross with the foundation stock shows a highly significant decrease in penetrance ($\chi^2 = 41.41$, $P < .001$), but no significant lowering of expressivity.

SUMMARY

In conclusion, it can be said that following crosses to Dorking fowl recessive rumplessness occurred in larger numbers of offspring, as far as all types of the abnormality are concerned (penetrance), and that incidence of complete rumplessness (expressivity) was significantly raised in comparison with the low degree of penetrance and expressivity in the original White Leghorn-derived stock. The opposite, at least as far as penetrance is concerned, was found in the descendants of crosses to Jungle fowl. These differences agree with what one might have expected if the innate tendencies of the two stocks to produce widely differing incidences of sporadic rumplessness and their similar divergence in response to rumplessness-inducing chemicals had had an influence on penetrance and expressivity of the recessive rumpless mutation. If similar evidence can be obtained with other material, it should become possible to test, and perhaps verify the hypothesis that the occurrence of sporadic malformations with typical stock frequencies and with characteristic strain differences in response to experimental conditions are brought about by hereditary factors which ordinarily are insufficient to interfere with normal development, but which may become a part of the hereditary mechanism for polyfactorially-transmitted traits.

LITERATURE CITED

Dunn, L. C., S. Gluecksohn-Schoenheimer, M. R. Curtis and W. F. Dunning, 1942, Heredity and accident as factors in the production of taillessness in the rat. Journal of Heredity 33: 65–67.

Dunn, L. C. and W. Landauer, 1934, The genetics of the rumpless fowl with evidence of a case of changing dominance. Journal of Genetics 29: 217–243.

1936, Further data on genetic modification of rumplessness in the fowl. Journal of Genetics 33: 401–405.

Landauer, W., 1945, Recessive rumplessness of fowl with kyphoscoliosis and supernumerary ribs. Genetics 30: 403–428.

Zwilling, E., 1942, The development of dominant rumplessness in chick embryos. Genetics 27: 641–656.

1945, The embryology of a recessive rumpless condition of chickens. Journal of Experimental Zoology 99: 79–91.

INTEGRATION OF THE GENE POOL AS DEMONSTRATED BY RESISTANCE TO DDT[1]

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For some time evidence has been accumulating that the gene pool of a population is not merely an agglomeration of particulate determinants but is an integrated whole having unique characteristics. Some rather striking data bearing on this point have come to light in the course of an investigation of the genetic nature of resistance to DDT in *Drosophila melanogaster*. Crosses between resistant lines developed independently from the same wild stock have given F_1's as resistant as the parent lines and F_2's significantly less resistant and with mortality distributions of apparently greater variances.

The two resistant lines (SyS-1001 and SyS-1002) were built up independently from the same wild stock, collected in Syosset, New York, in July, 1952, by treating adult flies with an aerosol of DDT dissolved in tri-n-butyrin and breeding the survivors. A series of treatments of varying duration was given in every generation and an LD_{50} in minutes of exposure was calculated. The survivors used as parents were flies from treatments where the mortality approximated 50 per cent and always numbered several hundred.

The measurement of resistance is unfortunately not precise. There is always considerable variation among repeated runs and the standard errors for all parameters are large. Hence there is fluctuation in the LD_{50} from generation to generation even when no real change has taken place. For this reason it is difficult to assign a precise LD_{50} to a given generation in a given line. Table 1 summarizes the pertinent data and gives a picture of the characteristics of the control, of the two selected lines, and of their behavior in the two crosses.

The most accurate procedure for analyzing a dose-mortality relationship is a regression equation on a log-probit plot calculated by the maximum likelihood method (Finney, 1952). The maximum likelihood calculations are elaborate and time-consuming and have been used only for the data from the crosses where maximum precision was desired. A simplified modification of this method, developed by Litchfield and Wilcoxon (1949), has been used for the data from the control and the various generations of each line. In numerous cases where the two methods have been applied to the same sets of data, the results have always been substantially the same, the simplified method usually giving somewhat larger confidence intervals.

[1]The work reported here has been done under contract No. DA-49-007-MD-327, Medical Research and Development Board, Office of the Surgeon General, Department of the Army.

The LD_{50} is the dose measured in minutes of exposure which kills 50 per cent of the treated flies. S, the slope function, is the antilog of the reciprocal of the slope (b) of the regression line. It is the fold change in dose necessary to produce a change in mortality equal to one standard deviation unit. S is therefore a function of the standard deviation of the mortality distribution.

TABLE 1

Line	Gen.	LD_{50}	95% Conf. Limits		S	95% Conf. Limits	
Sy (Unsel.)		5.3	4.6	6.2	1.94	1.67	2.25
SyS-1001	1	7.0	4.2	11.6	1.68	1.02	2.77
	15	21.6	18.3	25.5	2.22	1.89	2.60
	16	20.7	17.8	24.1	1.84	1.53	2.21
	17	18.0	14.9	21.8	2.37	1.78	3.16
	(X2) 18	17.5	14.7	21.1	2.17	1.66	2.84
	19	19.3	14.5	25.7	2.18	1.46	3.23
	20	15.5	12.1	19.8	2.13	1.54	2.93
	21	21.2	17.1	26.3	2.05	1.54	2.73
	22	27.0	23.1	31.5	1.94	1.60	2.34
	(X3) 23	26.5	18.7	37.6	2.10	1.29	3.42
SyS-1002	1	5.2	3.7	7.3	2.06	1.32	3.21
	14	18.4	14.7	23.0	1.96	1.56	2.47
	15	18.5	15.2	22.6	2.04	1.61	2.59
	16	15.8	13.6	18.3	2.29	1.85	2.84
	(X2) 17	21.5	17.1	27.1	2.29	1.57	3.34
	18	17.9	15.6	20.6	2.56	2.02	3.25
	19	20.0	15.6	25.6	2.23	1.58	3.14
	20	25.4	18.7	34.5	2.12	1.39	3.22
	21	27.2	22.8	32.3	2.16	1.64	2.82
	(X3) 22	25.2	21.9	28.9	2.23	1.81	2.74
X2	F_1	21.7	19.7	24.2	2.13	1.90	2.53
X2R	F_1	20.1	16.2	25.6	2.39	2.04	3.05
X2	F_2	10.2	8.3	12.4	2.88	2.32	4.18
X2R	F_2	11.0	8.1	14.4	2.42	1.95	3.63
X3	F_1	27.1	24.8	30.2	2.14	1.93	2.48
X3R	F_1	25.0	22.1	29.1	2.07	1.78	2.68
X3	F_2	14.8	12.3	17.7	2.65	2.14	3.87
X3R	F_2	14.2	12.8	15.8	2.35	2.05	2.77

From table 1 it may be seen that the LD_{50} of the unselected stock from which both lines sprang was 5.3 minutes. Later data for the control indicate that six minutes is probably a satisfactory estimate. When the first cross was made between the eighteenth and seventeenth generations, this figure had increased in the selected lines about threefold, to the neighborhood of about twenty minutes.

In the first cross (X2, SyS-1001♀♀X SyS-1002♂♂) and its reciprocal (X2R, SyS-1002♀♀X SyS-1001♂♂) the LD_{50}'s of the F_1 were not significantly different from each other nor from the figures for the lines crossed. In the F_2 the LD_{50}'s were cut approximately in half and were significantly lower than they were in the F_1 or in the two lines. Again, there was no significant difference between the two crosses.

At generations 22 and 23 of the two lines, when the cross was repeated, the LD_{50}'s had reached about four times the control figure, approximately twenty-five minutes. Here again the F_1's of the crosses are almost identical with the parent strains in LD_{50}. When we look at the F_2's, we see again a sharp and significant drop in the LD_{50}. Again there is no indication of difference between the reciprocal crosses.

Since the flies used in these crosses were not selected in the generations crossed, it might be suspected that the drop in the LD_{50} from the F_1 to the F_2 was the result of relaxing selection. This explanation, however, is not valid. At generation 19 a sample of SyS-1002 was placed in a population cage and allowed to breed without further selection. Tests run on the F_2 and F_3 of this sub-line gave no indication of a decline in resistance.

If one looks at the data on the two lines and on the F_1's of the crosses, one is tempted to conclude that the two selected lines are identical. Having been developed by the same type of selection from the same original stock, they have arrived at the same level of resistance. A cross between them gives offspring with the same degree of resistance. From this argument and conclusion one would expect to observe the same resistance in the F_2. How are we to explain the sharp and significant drop?

The only reasonable explanation is that the two lines are not identical genetically. Apparently there are different combinations of genetic factors which can result in resistant phenotypes. SyS-1001 has achieved resistance by consolidating certain of these; SyS-1002 has arrived at the same level of resistance by consolidating others. In the F_1 of a cross between them every individual carries one haploid set of chromosomes from each line. That such genotypes are not more resistant than the parental lines indicates that no simple dominance relationship exists. In the F_2 where the individual chromosome sets have various proportions of genetic material derived from each line, many individuals do not have a complete set of resistance factors from either line and hence their resistance is lower.

All other evidence accumulated in the course of the present investigation supports this hypothesis of the multifactorial or polygenic nature of the character of resistance. Resistance builds up slowly in every selected line. In no case has a significant difference been achieved in fewer than a dozen generations of selection. When response occurs, it comes by slow increments. And a high level of selective intensity is less effective in producing resistance than one based on 50 per cent mortality (King, 1954, 1954a). Crow (1954) has been led by his work to a similar conclusion. This evidence by no means proves that single genes for high resistance do not exist. Ogaki and Tsukamoto (1953) (Tsukamoto and Ogaki, 1953, 1954) report having found one in a natural population. Our evidence does, however, indicate that polygenic systems for resistance are probably more common.

If the drop in the resistance of the F_2 is the result of the segregation of factors in which the two lines differ, we should expect the decline to be accompanied by an increase in the variance of the mortality distribution.

TABLE 2

Line	Gen.	LD_{10}	95% Conf. Limits		LD_{50}	95% Conf. Limits		LD_{90}	95% Conf. Limits	
X2	F_1	8.2	6.8	9.8	21.7	19.7	24.2	57.3	44.9	74.4
	F_2	2.6	1.6	3.6	10.2	8.3	12.4	39.7	29.3	63.0
X2R	F_1	6.6	5.4	8.8	20.1	16.2	25.6	61.0	46.1	93.1
	F_2	3.6	1.8	5.1	11.0	8.1	14.4	32.3	24.3	52.9
X3	F_1	10.2	8.9	11.4	27.1	24.8	30.2	72.1	59.1	94.7
	F_2	4.2	2.6	5.5	14.8	12.3	17.7	51.6	43.4	61.6
X3R	F_1	9.9	6.7	13.1	25.0	22.1	29.1	63.4	49.0	96.7
	F_2	4.8	3.9	5.9	14.2	12.8	15.8	42.6	34.7	52.3

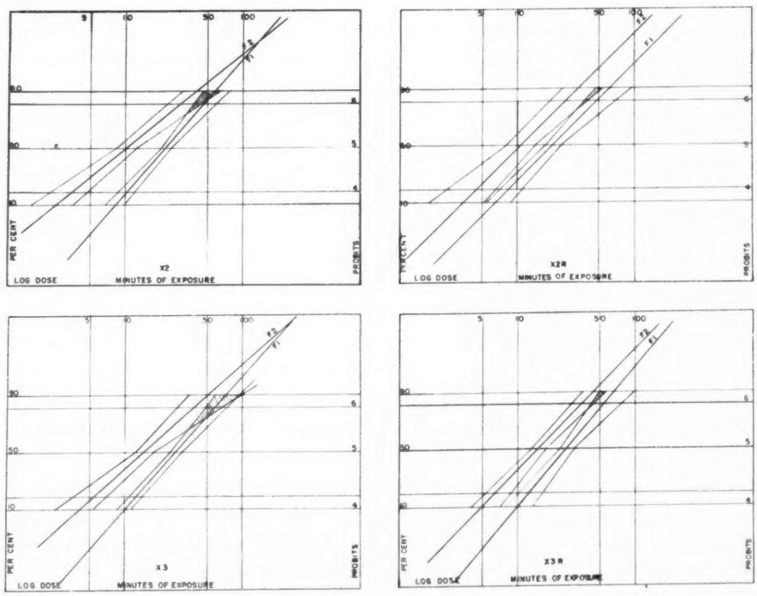

FIGURE 1. Graphs showing mortality regression lines and 95 per cent fiducial bands between LD_{10} and LD_{90} for the F_1 and F_2 of four crosses between two independently developed lines of *Drosophila melanogaster* resistant to DDT. Long diagonal lines are the mortality regressions as labeled. Shorter diagonals connect 95 per cent confidence limits at LD_{10}, LD_{50}, and LD_{90}. Overlap of fiducial bands cross-hatched.

Crosses: X2 (SyS-1001 F_{18} ♀♀ X SyS-1002 F_{17} ♂♂)
X2R (SyS-1002 F_{17} ♀♀ X SyS-1001 F_{18} ♂♂)
X3 (SyS-1001 F_{23} ♀♀ X SyS-1002 F_{22} ♂♂)
X3R (SyS-1002 F_{22} ♀♀ X SyS-1001 F_{23} ♂♂)

If we examine the data with this hypothesis in mind, we get no such clear-cut answer as when we compared the LD_{50}'s. Our measure of the variance of the distribution is the slope function, S. The steeper the regression line, the smaller is the variance and the smaller the value of S. Table 1 gives the S values and their 95 per cent confidence intervals for all the LD_{50}'s listed. The confidence intervals are large and there are no significant differences between the S values of the control and of any given generation in either selected line. If we look at the S values for the crosses themselves, we see that there are no significant differences between F_1 and F_2. However, within the cross data the S value for the F_2 is always higher than that for the corresponding F_1. The lowest S value for an F_2 is 2.35 (X3R). This is exceeded by only one F_1 value (X2R) and among the figures by generations of the two selected lines, by only two, SyS-1001 F_{17} and SyS-1002 F_{18}.

The data for the crosses may be presented in another way which shows the tendency toward a greater variance in the F_2. Table 2 gives the values and their confidence limits for the LD_{10}'s and the LD_{90}'s. Figure 1 shows the same thing graphically. Since the error of the slope of a regression line allows for its pivoting around the $\bar{x},\bar{y}$ point, the confidence intervals for values along the line increase as one goes toward the extremes. Thus the confidence intervals for the LD_{10}'s and the LD_{90}'s are larger than those for the LD_{50}'s. In all four crosses this increase results in an overlap of the confidence bands for F_1 and F_2 toward the upper end of the distribution including in all cases the LD_{90}. In none of the four cases is there an analogous overlap at the LD_{10}. If all the regression lines were really parallel, and these overlaps were merely the result of the heterogeneity of the data, they would be as likely to occur at the lower end of the distribution as at the upper.

DISCUSSION

Two populations, originally similar sub-samples of a single stock, have developed within about twenty generations under pressure of artificial selection two different genetic systems, both of which produce a modal phenotype different from that of the original stock. These two populations are phenotypically indistinguishable but are genetically different, and so far as resistance is concerned, ill adapted to each other. When crossed, they produce in the F_2 a modal phenotype more like that of the original stock.

The significance of this finding lies in the fact that it dove-tails very neatly with the results of a number of other recent experiments which, taken together, appear to offer a basis for a more complete understanding of population genetics and the evolutionary process. Without attempting an extended review of this complicated subject, it may be worthwhile to mention a few of these interlocking blocks.

It has long been known that the expression of a gene is subject to the influence of genetic modifiers and that a gene changes its expression in different genetic backgrounds. Multifactorial inheritance has been recognized for several decades. Mather's (1949 and earlier papers cited) work on poly-

genic inheritance has established that continuously varying phenotypes can be explained by multifactorial systems in which every individually segregating factor contributes an increment smaller than the range of developmental variation. Robertson and Reeve (1952, 1953; and Reeve and Robertson, 1953) have shown that when a polygenic system is altered by selection so that the modal phenotype is driven in a given direction, a conservative force tends to cause it to revert when selection is relaxed, that the contributions of the individual genetic factors are often non-additive and that these contributions are often radically altered when a change is made in the genetic background.

Dobzhansky (1950; Dobzhansky and Levene, 1951; Dobzhansky and Pavlovsky, 1953; and earlier papers cited) has shown that populations of several species of Drosophila are systematically polymorphic in chromosomal structure, that this polymorphism is maintained because of the adaptive superiority of structural heterozygotes, that this superiority is limited to individuals heterozygous for structurally different coadapted chromosomes from within the same local population; and, finally, that while synthetic populations containing structurally different chromosomes of diverse geographic origin show at first no adaptive advantage of the structural heterozygotes, such advantage may or may not develop within the course of a few months.

Wallace and co-workers (Wallace and King, 1952; Wallace *et al.*, 1952) have found that isolated laboratory populations of *Drosophila melanogaster* rapidly develop individually characteristic genetic systems which can be described by the results of genetic tests, that non-additive interactions between genetic factors are an important element in the production of modal phenotypes and that the interactions within a population differ from those between factors from different populations.

Vetukhiv (1953) has demonstrated that F_1 hybrids between different natural populations show an increase in viability as compared with the parent strains, but that the F_2 individuals display a decrease in viability to a point lower than that of the parent strains. Waddington (1953), starting with a wild-type population, has by means of artificial selection built up a polygenic system producing a phenotype indistinguishable from that characteristic of a given homozygous mutant. Working from an entirely different point of view, Mayr (1953) has pointed out that peripheral populations of many species of birds seem to be restricted in phenotypic variability as long as they are tied to the central population by interbreeding, but often display sudden and striking changes in phenotype when they become isolated from the gene pool of the whole species.

All these diverse observations point quite clearly in the same direction. They emphasize that the genetic material of a population of cross-fertilizing organisms is not a fortuitous aggregate of particulate units, each determining its proper part of the phenotype in isolation from the others, but is an integrated whole in which the elements work in concert. Any change in the proportions of different genetic elements, whether produced by selection or

by migration, requires compensatory changes in other elements to produce a newly integrated equilibrium. This seems to be precisely what has happened in the two selected lines resistant to DDT. The reduction of those factors most frequently found in susceptible individuals induced adjustments in the remainder of the genetic system. The two lines made these compensatory readjustments in different ways indicating that there are, very likely, numerous systems of readjustment possible. Two samples from the same population can develop distinctly different genetic systems within as few as twenty generations; all the evidence is that local populations of most species also have distinct genetic differences. It seems reasonable to infer that, in general, the genetic system of a population is in dynamic equilibrium and is capable of rapid, complex readjustments. The notion often encountered that two species may differ in only one or in a small number of pairs of alleles becomes very difficult to maintain. We have observed an illustration of what Simpson (1949) has called "the opportunism of evolution." He cited it in ages of natural history of antelope horns; it can also be seen in the laboratory within a time-span measured in months.

SUMMARY

Crosses between resistant lines of *D. melanogaster*, developed independently by the same method of selection over some twenty generations, give an F_1 population of the same resistance as the parent lines and an F_2 with significantly lower resistance and a mortality distribution of greater variance. These phenomena indicate that the two lines have achieved resistance by consolidating different combinations of factors for resistance. The significance of this finding is discussed in the light of other recent evidence that the gene pool of a population is an integrated whole.

LITERATURE CITED

Crow, J. F., 1954, Analysis of a DDT resistant strain of Drosophila. Jour. Econ. Entom. 47: 393-398.

Dobzhansky, Th., 1950, Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics 35: 288-302.

Dobzhansky, Th., and H. Levene, 1951, Development of heterosis through natural selection in experimental populations of *Drosophila pseudoobscura*. Amer. Nat. 85: 247-264.

Dobzhansky, Th., and O. Pavlovsky, 1953, Indeterminate outcome of certain experiments on Drosophila populations. Evolution 7: 198-210.

Finney, D. J., 1952, Probit analysis. 2nd edition. The University Press, Cambridge, England.

King, J. C., 1954, The genetics of resistance to DDT in *Drosophila melanogaster*. Jour. Econ. Entom. 49: 387-393.

1954a, The genetics of resistance to insecticides. Ann. Rep. Biol. Lab. 64 (1953-1954): 39-43.

Litchfield, J. T., Jr., and F. Wilcoxon, 1949, A simplified method of evaluating dose-effect experiments. Jour. Pharm. and Exp. Therap., 96, No. 2: 99-113

Mather, K., 1949. Biometrical genetics. Dover Publications.

Mayr, E., 1953, Change of genetic environment and evolution. In "Evolution as a process," J. Huxley, Ed. Allen and Unwin, London, England.

Ogaki, M., and M. Tsukamoto, 1953, Genetical analysis of DDT resistance in some Japanese strains of *Drosophila melanogaster.* Botyu Kagaku 18: 100–104. (English résumé, 103).

Reeve, E. C. R., and F. W. Robertson, 1953, Studies in quantitative inheritance. II. Analysis of a strain of *Drosophila melanogaster* selected for long wings. Jour. Genet. 51: 276–316.

Robertson, F. W., and E. Reeve, 1952, Studies in quantitative inheritance. I. The effects of selection of wing and thorax length in *Drosophila melanogaster.* Jour. Genet., 50: 414–448.

Robertson, F. W., and E. C. R. Reeve, 1953, Studies in quantitative inheritance. IV. The effects of substituting chromosomes from selected strains in different genetic backgrounds in *Drosophila melanogaster.* Jour. Genet., 51: 586–610.

Simpson, G. G., 1949, The meaning of evolution. Yale University Press, New Haven, Conn.

Tsukamoto, M., and M. Ogaki, 1953, Inheritance of resistance to DDT in *Drosophila melanogaster.* Botyu Kagaku 18: 39–44 (English résumé, 43).

1954, Gene analysis of resistance to DDT in *Drosophila melanogaster.* Botyu Kagaku 18: 25–32 (English résumé, 31).

Vetukhiv, M., 1953, Viability of hybrids between local populations of *Drosophila pseudoobscura.* Proc. Nat. Acad. Sci. 39: 30–34.

Waddington, C. H., 1953, Genetic assimilation of an acquired character. Evolution 7: 110–126.

Wallace, B., and J. C. King, 1952, A genetic analysis of the adaptive values of populations. Proc. Nat. Acad. Sci. 38: 706–715.

Wallace, B., *et al.*, 1952, An analysis of variability arising through recombination. Genetics 38: 272–307.

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

EFFECTS OF PORCUPINE QUILLS IN HUMANS

The penetration of porcupine quills into the human body is never a pleasant sensation, but twenty years of experience in working with a porcupine colony, and continued handling of these spiny animals, have convinced the author that description of the discomfort of being quilled is often very much exaggerated. It has been described as a "horrible sensation" with every quill a "fiery dart" in the flesh. During the years of experience, many hundreds of quills have penetrated various parts of the author's own body in numbers of one or two, to as many as forty at one time. Usually the fingers, hands, or arms were the areas quilled, but on one occasion forty were driven into the forehead and bridge of the nose by one stroke of a porcupine's quill-studded tail, but glasses prevented any injury to the eyes. Even the large number of quills which entered the flesh of that sensitive region and penetrated the periosteum of the bone, did not cause very severe pain except the first momentary flash at the instant when the many spines were driven into the face.

The second dominant sensation experienced was like wearing a heavy mask which completely immobilized the area. *Pulling the quills out*, one at a time, is *very painful unless* done with a quick movement which *jerks the quill straight back* in the opposite direction from which it entered the flesh. By jerking it straight backward there is also less danger of breaking off the point, which when left in the flesh, travels farther into the body. The instantaneous removal also reduces to a minimum the attendant discomfort of quill removal.

When the grasp of the thumb and finger is not strong enough to jerk out the quills, flat jawed pliers make a very efficient retractor, but care should be taken not to press hard enough with the pliers to break or cut the spine in two.

The immediate removal of the quills from the face and forehead took approximately four minutes and caused a minimum of discomfort. The major part of the sharp pain occurred (1) during the quilling and (2) in the process of extraction. Over the first half-hour, the nose and the middle of the forehead developed a marked feeling of gradual stiffening in the flesh, with a slight amount of soreness developing in the deeper parts penetrated. At times, a warm flush in the region quilled was experienced, but at no time was there a "burning or fiery" sensation or any "excruciating pain," as described by some writers. After the short interruption of the spine removal, the evening laboratory observations were resumed and

completed with little thought of the experience. Slight rubbing of the injured region gave some relief and it was swabbed once with 70 per cent ethyl alcohol.

An hour after being quilled, the stiffness was quite marked and the development of soreness was more evident, particularly when pressure of the finger was applied to the points of injury. Twelve hours later, the stiffness was almost entirely gone but there was a definite soreness to the touch, especially in the deeply penetrated flesh. Within four days the soreness gradually abated, although no treatment was used except the one swabbing of alcohol. No infections developed.

Cases of deep penetration in the sensitive finger tips and/or the palms of the hand, are likely to be more painful, i.e., the soreness is more pronounced and of longer duration than in areas on the arms and legs. Deep wounds in these more sensitive regions may be accompanied first by an acute stabbing pain, followed by pronounced aching sensations, which may be accompanied, or followed by some stiffness and rather more persistent soreness than in most other areas.

Penetration of quills near joints of fingers gave more trouble than in any other region. In one quite painful experience, in which the tip of a quill broke off near the basal joint of the right index finger, the stiffness and soreness became so acute that the grip of the right thumb and finger was completely incapacitated for several weeks, and the use of the hand was partially impaired for approximately five months, but its use gradually returned. The quill fragment was never recovered.

Migration of a whole spine, or fragment of the point in the sensitive tip of a finger may prove quite uncomfortable, particularly when pressure is applied by grasping something, but in less sensitive muscular areas, e.g. the arm, the progress of the fragment is not really painful (Shadle, 1947)[1]. During the migration, one occasionally experiences for a moment a slight pricking sensation which alerts one to the progress of the fragment in the tissues, but it is really not uncomfortable for the sensation lasts but an instant. If the fragment happens to come to rest under a finger nail it may have to be removed or it will at least need aid in making its escape from the tissues.

Although quills in other animals often are the cause of infections this is not particularly true of humans. In the lower animals, they are seldom able to remove the spines while in humans they are soon extracted and there is less probability of infection developing. Only twice did the author experience infections, once in the joint of the finger and once when a broken fragment passed dorsad through a finger tip and came to rest against the underside of the fingernail.

In comparing injuries of humans from deep long splinters of wood with injuries from porcupine quills, the author would quite definitely prefer to deal with the quills rather than with the splinters. First, the spines are usually larger and more easily handled. Second, removal of the quill is ordinarily easily and quickly accomplished, and this is seldom true with

splinters. Third, comparing the same number of injuries from splinters with the same number from quills, the incidence of infections from splinter wounds would be much higher than quill infections which amount to a small fraction of one per cent.

LITERATURE CITED

[1]Shadle, Albert R., 1947, Porcupine spine penetration. Jour. Mammal., 28: 180-181.

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SECONDARY CENTRIC ACTIVITY IN LILIUM FORMOSANUM

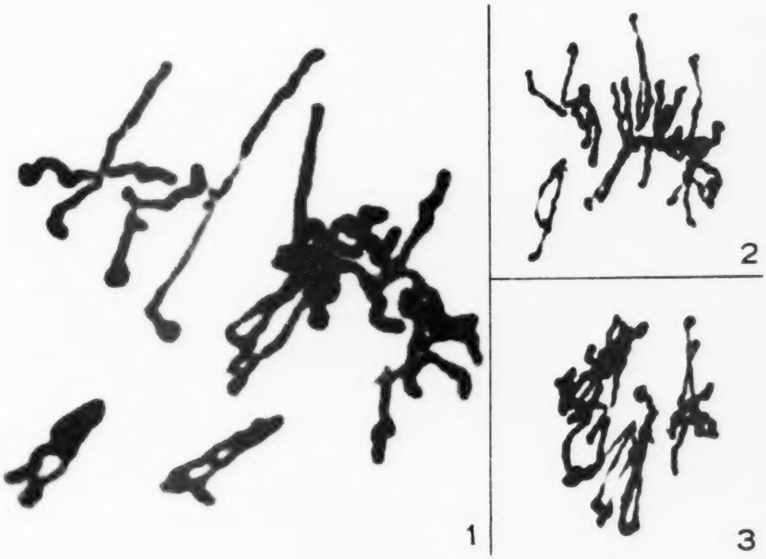
Centromere-like activity during meiosis of chromosome regions other than the centromere has been observed recently in several members of the grass family. Prakken and Müntzing[1] described such activity as "T phenomena" in rye, and Rhoades and Vilkomerson[3] reported on similar behavior in corn, first as "secondary centric" activity and later (Rhoades[2]) as "neo-centric regions." Similar behavior has been reported by Vilkomerson[4] in *Elymus Wiegandii* and by Walters[5] in a Bromus hybrid: *B. pitensis* × *B. marginatus*. Usually the clearest manifestation of this activity is in second metaphase where terminal or subterminal regions (which were identified as corresponding to knobs in corn) were observed to be attracted, apparently at random, towards the poles while the centromeres were yet undivided and still oriented on the metaphase plate.

Similar kinetic activity in metaphase II was found to occur in three plants of *Lilium formosanum*. Two plants were found during a routine examination of meiosis (aceto-carmine smear technique) in X_1 plants (1000 r pollen treatments). Another case was detected among a group of control plants. Numerous other plants examined have shown normal metaphase II behavior. Of the two X_1 plants, one had an X-ray induced paracentric inversion and the other, although not showing any apparent chromosomal aberration, had 50 per cent pollen abortion.

Secondary centric behavior in the three plants was very similar. In each of them, this activity was not restricted to any specific chromosome. Any one of the 12 chromosome dyads showed the activity at metaphase II in one cell or another. The active regions were usually subterminal (figs. 2 and 3). The two homologous chromatids in each dyad have been observed to be attracted either both to the same pole or each to a different one, thus indicating a lack of opposite polar orientation of the two homologous active regions. When the homologous chromatids are attracted to one pole, sometimes the whole dyad is observed to have moved outside the metaphase plate in the direction of that pole (fig.1). For any given chromosome dyad the manifestation of the secondary centric activity was different from cell to cell. In some cells only a single chromatid was observed to be active in the dyad, in other cells two chromatids were stretched and in still other cells (especially in cases of the metacentric chromosomes) three chromatids or all four of them showed the attraction. This secondary attraction is apparently effective only in metaphase II and early anaphase II since late anaphase II cells show normal arrangement of chromosomes. Very large variation existed in the degree of secondary centric activity. In all three plants some cells (such as in the figures) showed the attraction very clearly while other cells, on the same slide, had only a weak manifestation of this activity or none at all.

To exclude the possibility that the phenomenon described was a result of stretching and dislocation caused by the smearing technique, several slides

have been examined before the application of the cover slips. The secondary meiocytes were dissected out of the anther in a drop of aceto-carmine, and the cells were examined under high-dry magnification while still floating in the drop. The same picture of chromatid arms stretched towards the poles, and of dyads off the equatorial plate was observed in these cells, indicating that the figures were not artifacts due to smearing.



ACETO-CARMINE SMEARS OF SECOND METAPHASE CELLS SHOWING PRONOUNCED SECONDARY CENTRIC ACTIVITY.

FIGURE 1. (X 1140). Two dyads lie at the bottom of the figure off the equatorial plate and show two homologous chromatids pushing towards the same pole. The ordinary centromeres are clearly visible in the three dyads at the left, the first of which has a median centromere while the other two have subterminal centromeres.

FIGURES 2 and 3. (X 600). Cells are somewhat less flattened than that of figure 1. The subterminal position of the active regions is visible in most of the stretched chromatids.

The observation of secondary centric activity in *Lilium formosanum* indicates that this meiotic peculiarity is not restricted to the Graminae. One might therefore suspect that this phenomenon may be of much more general significance than has been previously believed.

ACKNOWLEDGEMENT

The author wishes to thank Dr. Spencer W. Brown for his advice and criticism.

SUMMARY

Kinetic activity, during meiotic metaphase II, of chromosomal regions other than the centromere, has been observed in *Lilium formosanum.* This behavior is similar to the "secondary centric" activity reported recently in several species of the grass family.

LITERATURE CITED

[1]Prakken, R., and A. Müntzing, 1942, A meiotic peculiarity in rye, simulating a terminal centromere. Hereditas 28: 441–482.

[2]Rhoades, M. M., 1952, Preferential segregation in maize. In *Heterosis*, edited by J. W. Gowen, Iowa State College Press, Ames, Iowa: 66–80.

[3]Rhoades, M. M., and H. Vilkomerson, 1942, On the anaphase movement of the chromosomes. Proc. Nat. Acad. Sci. 28: 433–436.

[4]Vilkomerson, H., 1950, The unusual meiotic behavior of *Elymus Wiegandii.* Experimental Cell Research 1: 534–542.

[5]Walters, Marta Sherman, 1952, Atypical chromosome movement in meiotic anaphase of *Bromus pitensis* × *B. marginatus.* Amer. Jour. Bot. 39: 619–625.

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A NEW METHOD OF COLLECTING DROSOPHILA BY MEANS OF STERILE BAIT

In surveying the population of *Drosophila* in a certain region it is customary to attract the flies to a bait consisting of mashed autoclaved banana fermented by bakers yeast. If the purpose of the survey is to obtain information on the microbial flora upon which the adult fly population feeds, this method has certain disadvantages. It is difficult under field conditions to prevent entirely that the flies gain access to the bait. A procedure which is often used is to place a fine metal screen over the bait or to catch the flies before they have an opportunity to feed. In spite of such precautions, Shehata and Mrak[1] in a recent survey, found that 27 of 118 yeasts isolated from the intestinal tract of flies collected in certain mountain and desert regions of Southern California were typical bakers yeasts. However, 52 yeasts isolated from a variety of natural plant materials in the same areas did not include bakers yeast. In a similar survey by the present author in the Northern California mountains 74 yeasts isolated by the above method yielded 12 isolates of bakers yeast, but none were obtained from local vegetative sources. This strongly indicates that the flies were able to reach the bait in spite of the precautions taken.

It was felt that a solution to this problem should be sought in a cold sterilization of the bait, since autoclaving is likely to destroy at least part of the labile volatile compounds responsible for attraction. Ethylene oxide and propylene oxide are compounds which are now used extensively for the cold sterilization of certain foods[2] and biological materials[3], 4. When added to properly fermented banana bait, yeasts, bacteria and molds (including their spores) are readily killed. Since the epoxides are very unstable in acidic aqueous media, they decompose in a few days into their nontoxic corresponding glycols.

The procedure which was adopted is as follows. Well fermented banana bait was homogenized in a Waring blendor. A measured volume is placed in a screw cap jar and with a refrigerated pipet one percent by volume of ethylene oxide is added beneath the surface of the mash. The mixture is then stirred rapidly and thoroughly and allowed to stand at room temperature for two or three days in the tightly closed jar. During this period the yeast is killed and the epoxide decomposes. The bait is then ready for use. Since ethylene oxide has a low boiling point (10.4°C) it may be more convenient in some laboratories to use propylene oxide (B.P. 35°C), although it is a slightly less effective germicide than ethylene oxide. The flies may be permitted to feed freely on the treated bait and the extended crops facilitate subsequent dissection prior to streaking the crop contents on an agar medium. By the use of the described procedure, several hundred yeasts have been isolated from wild *Drosophila* flies, without encountering a single isolate of a typical bakers yeast.

LITERATURE CITED

[1]Shehata, A. M. El Tabey A., and Mrak, E. M., 1952. Intestinal yeast floras of successive populations of *Drosophila*. Evolution 6: 325–332.

[2]Whelton, R., Phaff, H. J., Mrak, E. M., and Fisher, C. D., 1946. Control of microbiological food spoilage by fumigation with epoxides. Food industries 18: 23–25.

[3]Wilson, A. T., and Bruno, P., 1950. The sterilization of bacteriological media and other fluids with ethylene oxide. Jour. Exptl. Med. 91: 449–458.

[4]Hansen, H. N., and Snyder, W. C., 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37: 369–371.

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BOOK REVIEW

THE COMING OF AGE OF BLOOD GROUP RESEARCH

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The appearance of two books* at the end of August 1954 marks a definite stage in the development of a field which can now be seen in perspective as one of the important research instruments in the study of human evolution. The starting point, Landsteiner's discovery of the four blood groups, coincided in time with the beginning of modern genetics in 1900. Blood group serology and genetics have grown up together and in increasingly close connection. Bernstein's interpretation in 1924 of the genetical system responsible for the heredity of the ABO groups gave the impetus to the merging of the two fields of research, of which we now see the fruits. The Hirzfelds in 1918 laid the foundation of blood group anthropology by their demonstration of differences in the frequency of the four Landsteiner blood types in racially different populations. The Boyds, W. C. and L. G., exploited and expanded this lead, and W. C. Boyd's compilation of 1939 and his discussion of 1950 (Genetics and the Races of Man) showed that a new chapter could be added to racial anthropology. The blood groups came into their own in fields related to medicine such as serology, physiology, hemotology and pathology largely as a result of the discovery in 1940 of the Rh system and of its connection with hemolytic disease of the newborn by Landsteiner, Wiener, and Levine. The dramatic development and expansion of the new field thus opened has been due in the first instance to Wiener and Levine, to the vigorous technical and theoretical work of a group of British investigators, of which the authors of the two new books cited have been leading representatives, and to active teams and individual workers in every one of the continents.

Not the least of the influences responsible for the present healthy state of affairs in this field has been the development of two foci of cooperative international research at the Lister Institute in London under the aegis of the British Medical Research Council. One of these, The Blood Group Reference Laboratory, is directed by Dr. Mourant, the author of the first of the new books to be discussed; the other, The Blood Group Research Unit, is directed by Dr. Race who with his collaborator, Dr. Ruth Sanger, has just published the second revised edition of a book first published in 1950. The new edition will be briefly noted below.

*Mourant, A. E., The Distribution of the Human Blood Groups. Blackwell Scientific Publications, Oxford. 42 shillings.

*Race, R. R., and Ruth Sanger, Blood Groups in Man, Second Edition. Blackwell Scientific Publications, Oxford. 30 shillings.

What one now sees setting in is a rising tide of research on a world-wide front participated in by medical researchers, transfusion officers, blood banks, serologists, hematologists, anthropologists, statisticians, and theoretical biologists. The two books bear very clearly the marks of individual authorship and responsibility; yet equally clearly they derive from the voluntary, informal and personal relationships amongst a growing body of investigators, whose results, published and unpublished, have been collated, criticized, and pondered by the authors. The result is an assessment of the present state of blood-group research which is as broad, inclusive, complete and authoritative as could be expected in so active and rapidly changing a field.

The book by Dr. Mourant is the first complete compilation of the geographic and racial variation in the frequencies of the genes comprising the main blood group systems, of the genes for thalassemia (also called microcytemia or Mediterranean anemia), sickle-cell trait and disease (also called falcemia or drepanocytosis) with some observations on other genes of anthropological interest, such as that governing secretion of ABH blood group substances, ability to taste phenylthiocarbamide, and a few others. The descriptive part of the book replaces Boyd's compilation of 1939 which brought together the literature through 1938 on the distribution of the ABO, MN and P groups.

The major changes which have occurred since that time, as noted in this book, are as follows: 1. Six new blood group systems useful in anthropological classification have been discovered (the Rh, Lewis, Lutheran, Duffy, Kell, and Kidd Groups). The MN system has been expanded by the discovery of the S, s, and Henshaw antigens belonging to it, and the Hunter antigen of Landsteiner and Chase has been shown to belong to it. Several new varieties of the sickling gene and the chemical nature of the hemoglobins affected by it have been investigated.

2. There has been a great expansion of the data on the geographic and racial variations in the frequencies of the older systems, and on the worldwide distribution of the genes of the more recently discovered systems, of sickling, and, particularly in the Mediterranean region, of thalassemia.

3. Serious evidence has appeared that one of the factors responsible for variations in the frequencies of some of the genes listed above is selection. At the time of Boyd's review, and for most of the time since, random fluctuation of gene frequencies in small populations (genetic drift) was the only factor which could be suggested in explanation of these variations in frequency. The new evidence has tended to emphasize selection as one of the chief agencies responsible for changes in the gene frequencies. This is a major change of outlook which requires a thorough-going reassessment of the evolutionary dynamics of all blood groups and similar factors and of their use in racial anthropology.

Dr. Mourant has set forth the data up to the early part of 1954 thoroughly and well. He has not attempted to treat the serology or the genetics of blood group systems in detail since this is done in the book of Race and

Sanger who in their turn do not discuss at length the geographic distribution or the questions of evolutionary and anthropological interest. In this sense the two books complement each other. The main emphasis in Mourant's book is to set forth the basic information on distribution. This is done in 40 tables covering 82 pages giving the original data with references to sources. The bibliography gives full citations of 1716 publications additional to those cited or quoted by Boyd, and is a chief and valuable feature of the book. The extent of some of the new knowledge is indicated by the table on the distribution of the sickle-cell trait which contains diagnoses of nearly 95,000 persons belonging to some 280 populations or tribes. Most of the observations were published in the last four years.

While not hesitating to make judgments concerning the reliability of the serological observations wherever the indications for or against are clear, Mourant tends to give those who report the data the benefit of any doubts which exist, apparently believing that tolerance is likely to produce more data to which time will apply the corrective. He has been more concerned to present an account of what appears at present as a great jigsaw puzzle picture in process of forming itself upon the map of the world, than to say what it means as a whole. His purpose in fitting the pieces together is to serve a new form of historical research. The ultimate problem is how the geographic varieties of man are related to each other in objective serological and genetical ways. Two main patterns appear on two different scales. One which might be called the macroscale is the indication of similarities of the peoples of great areas of continental dimensions, such as the African character of the Rh blood types, chiefly the chromosome cDe (Rho) of most of the natives of that continent south of the Sahara. This, he speculates, might represent the most primitive or ancestral Rh combination of genes. Similarly there is the virtual absence of ABO types other than O and the uniform frequencies of MNSs and Rh groups among all the aboriginal peoples of America.

On the other or microscale is the pattern of smaller areas, such as the shores of the Mediterranean Sea of which he has made a special study; of Wales as studied so fully for the ABO groups by Watkin; or of the small relict populations like the Basques or Lapps which in the extreme frequencies they present are of great interest not only in themselves, but as contributors to more extended populations and as evidence of previous conditions and movements of peoples. The contributions to human history which can come from intensive studies of small isolated populations, and of extensive surveys of large areas, when considered in the light of other historical archeological and ethnological evidence are clearly suggested in a most stimulating way, but without drawing categorical conclusions. In fact, the position which might have been occupied by a final chapter entitled "Conclusions" is represented by a single sentence from Francis Bacon's essay of Despatch: "I knew a wise man that had it for a by-word,

when he saw men hasten to a conclusion, 'Stay a little, that we may make an end the sooner'."

But if conclusions are withheld, judgments are not, and some of these are of great value for future research, and of special interest to students of human evolution. In a chapter on "An Attempt at a Synthesis," there are the following pregnant paragraphs; "It is particularly important to realise that random fluctuations can only operate on small populations, whereas selection acts just as fast on large as on small ones, though it is, in fact, a very slow process. The general uniformity of blood-group distribution over large populations and, even more, the close resemblance between populations known to have a common origin but to have been separated for many hundreds of years, tend to support the generally accepted hypothesis that a common origin between peoples leads to a long-maintained similarity in their blood-group distributions. The question is, how long?

"If the main cause of polymorphism is random fluctuation, then similarity in gene frequencies is strong evidence for the common origin of populations, even if they are known to have been separate for thousands of years. If, on the other hand, polymorphism is controlled by natural selection, similarity in gene frequencies may reflect convergence due to similar environments. Similarity may of course also be due to chance, but while it is to be expected that there will be many cases of chance resemblance in the frequencies of the genes of a single system, more complete chance resemblances affecting many systems are highly improbable.

"The relatively uniform frequencies of most of the blood-group genes over large areas of continental dimensions imply a long-term stability not closely dependent on the very varied external conditions provided by any such area. It is, at first sight, surprising to find that the frequencies of the Rh groups, subject as they are to selection by death from haemolytic disease of the newborn, show this kind of uniformity. The ABO groups are believed to be much less subject to selection by haemolytic disease, yet their frequencies vary widely over much smaller distances. This variation against a stable background of the genes of other systems, suggests that the ABO genes are relatively intensively selected by the environment. The discovery of a connection between blood groups and cancer shows that such selection can occur."

Coming from one whose wide knowledge of the subject and judicious temper are evident throughout the book such an opinion has a value exceeding that of speculation. It states in reasoned form an important concept which is fundamental in population genetics, namely, that the evolutionary effects of genetical variations have to be evaluated not merely by the influence on fitness of a particular allele or system, each one separately in context, but by the role which they play in a general mechanism such as the maintenance of polymorphism. When this reasoning is applied to the balance which appears to operate on the frequencies of the sickling gene, that is, to decrease in frequency by the adverse selection against homo-

zygotes dying from anemia, and to positive selection of heterozygotes in areas subject to subtertian malaria, it might have been extended one step further by pointing out that another element in the balance is the relative frequency of certain other genes. It is a fact, not mentioned by the author, that persons heterozygous both for the sickling gene and for the thalassemia gene often and perhaps usually, suffer from an anemia as severe as that which afflicts either of the homozygotes. Where the frequency of both of these genes is high, as in parts of Sicily as studied by Silvestroni and Bianco, adverse selection against each is increased by lethality in double heterozygotes. The view reached in experimental population genetics, that what is subjected to selection is the genotype as a whole, that is, the system of genes in combination and interacting, has still to be taken account of in blood-group research.

It is perhaps worth noting that the author's position with respect to the sensitivity of the ABO groups to selection has been strikingly validated by the appearance since the publication of the book of evidence that persons of blood group O in Great Britain run a risk some 35 per cent greater than persons of other blood groups of being hospitalized for peptic ulcer*.

It is likely that the effect of this book will be both wide and long. Many students of evolution will find in it a wealth of material perhaps beyond what they had expected. Anthropologists will recognize that the methods and results of gene frequency studies can be used to supplement the tested techniques of physical anthropology if used, as they are in this book, in conjunction with, rather than as replacements for, the classical methods. The way is now open for the enrichment of the systematics of man in much the same way that animal and plant taxonomy have been affected by the development of population genetics. Serology will be reciprocally benefitted by the ability to submit judgements and methods of the laboratory to a wider statistical court of appeals in which the validity of evidence can be tested by reference to unitary concepts arising from the proved connection between the gene and serological specificity. Finally, the emergence of a rigorous discipline of human biology, toward which this book is aimed, has by this clear objective consideration of a great body of new facts about man, been brought appreciably nearer.

Less needs to be said about the book of Race and Sanger since it follows the lines already laid down in the first edition which quickly established it as the standard work on the serology and genetics of the blood groups. For each blood-group system the essential facts of serological specificity, heredity as established from family data, methods of testing and of computing gene frequencies are given, and two new chapters, one on the Kidd groups and one on linkage, have been added. The latter records the first two autosomal linkage groups marked by blood-group genes, one containing genes for the Lutheran and Lewis antigens (Mohr, 1951), the other containing the loci of the Rh system and elliptocytosis (Lawler and

*Aird et al., Brit. Med. Jour., August 7, 1954.

others, 1953 and 1954), together with a conspectus of some twenty-five other genes which have been tested for linkage with some blood-group genes, none of them with convincingly positive results. The strong point of the earlier book remains; it is the critical examination of the basic serological facts and the perspicacity of the authors in seeking out the essential problems. Surely the ability to identify nine or even six blood-group antibodies in one serum as reported here is evidence of a kind of serological virtuosity upon which the identification of new antibodies and new antigens depends, as for example the recently discovered f of the Rh system. It is upon a basis of this sort that the future development of this field must rest. Questions of the genic structure of loci such as Rh and MNS, whether they are divisible by crossing over, as discussed here, or subject to position effect, are pertinent chiefly in the superstructure which can now be seen to be forming upon the serological foundation.

These books, by subsuming a great mass of new facts about human genes, which already begins to show great internal consistency, indicate that this field of evolutionary study is coming to a fruitful maturity.

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

Editorial Office, The American Naturalist
Box 2, Schermerhorn Hall, Columbia University
New York 27, N. Y.

Advances in Genetics, Vol. VI, 1954, Edited by M. Demerec. pp. 485, $9.50, Academic Press, New York.

The sixth volume of this annual publication contains a varied fare for those interested in heredity, evolution, agriculture and medicine. The articles are as follows:

"Map Construction in *Neurospora Crassa*" By Raymond W. Barratt, Dorothy Newmeyer, David D. Perkins and Laura Garnjobst. Text—pp. 85; Bibliography—pp. 8; "Genetic Changes in Human Populations, Especially Those Due to Gene Flow and Genetic Drift" By Bentley Glass. Text—pp. 40; Bibliography—pp. 5; "Monozygote Twins in Cattle" By John Hancock. Text—pp. 34; Bibliography—pp. 7; "The Genetics of the Newer Human Blood Factors" By Philip Levine. Text—pp. 43; Bibliography—pp. 7; "Comparative Incompatibility in Angiosperms and Fungi" By D. Lewis. Text—pp. 46; Bibliography—pp. 4; "Cytoplasmic Inheritance in Epilobium and its Theoretical Significance" By P. Michaelis. Text—pp. 106; Bibliography—pp. 8; "The Genetics of *Colias* (Lepidoptera)" By Charles L. Remington. Text—pp. 44; Bibliography—pp. 4; "Artificial Insemination and Livestock Improvement" By Alan Robertson. Text—pp. 19; Bibliography—pp. 2.
There is an author index and a subject index.

Barth, Lester G., and Lucena J. Barth, 1954. The energetics of development. 118 p. $3.00. Columbia University Press, New York.

Brown, Spencer W., 1954. Mitosis and meiosis in *Luzula campestris* DC. Vol. 27, No., pp. 231-278 in the University of California publications in botany. 12 figures, and 1 chart. $.75. University of California Press, Berkeley, California.

Coe, Wesley R, 1954. Bathypelagic nemerteans of the Pacific Ocean. Vol. 6, No. 7, pp. 225-286 in Bulletin of Scripps Institution of Oceanography. 32 text-figures, 2 plates. $.75. University of California Press, Berkeley, California.

Cole, William H. (Editor), 1954. Serological approaches to studies of protein structure and metabolism. 97 p., ill. $2.00. Rutgers University Press, New Brunswick, N. J.

Concise papers presented at the 1954 annual conference on protein metabolism at Rutgers University by A. A. Boyden, Melvin Cohn, David Gitlin, Felix Haurowitz, Michael Heidelberger, and J. Munoz.

Condry, William, 1954. Thoreau, 114 pp., frontispiece photograph. $3.50. The Philosophical Library, New York.

A brief biography and appreciation of the great observer, poet, and essayist of nature by a British critic and ardent Thoreauvian, printed in Great Britain to celebrate the 100th anniversary of the publication of Walden. This is an admirable brief account of the life and work of an unpopular man who became the literary ancestor of the popularizers of nature, none of whom reached the height or breadth of this peculiar Yankee who travelled so widely in Concord and in himself that he discovered things new and unique in the nature of man. The author wisely lets Thoreau speak for himself. Who else would ever make these sentences?

"I do not consider other animals brutes in the common sense. I am attracted towards them undoubtedly because I never heard any nonsense from them."—"If you are afflicted with melancholy—go to the swamp and see the brave new spears of skunk-cabbage buds already advanced toward a new year. Do they seem to have lain down to die, despairing of skunk-cabbagedom."

This little book which can be read through at a sitting may help many a perplexed naturalist of 1954 to glimpse other possible lives than those of "quiet desperation" which Thoreau found all about him; Even though they do not follow him "out to these solitudes where the problem of existence is simplified," nor read "not *The Times* but the Eternities," nor respond to his exhortation to Civil Disobedience, they still may find it hard to forget, after they have read him, that there are "woods bathed in so pure and bright a light as would have waked the dead, if they had been slumbering in their graves, as some believe. There needs no stronger proof of immortality. All things must live in such a light."

L. C. DUNN

Fogg, G. E., 1953. The metabolism of algae. 150 p., ill. $2.00. Methuen's Monographs on Biological Subjects. Methuen and Company, Ltd., London; John Wiley and Sons, Inc., New York.

The monograph on the metabolism of algae by G. E. Fogg is a succinct and authoritative review of recent advances in algal physiology and biochemistry. In this small volume of 150 pages are clearly summarized the subjects of oxidative metabolism, photosynthesis, assimilation of carbon and nitrogen compounds, nitrogen fixation and products of metabo-

lism of the algae. Lastly, there is a valuable chapter on algal growth and metabolism. This book is no mere compilation of references, although an excellent bibliography of over 300 pertinent references is provided. In the opinion of the reviewer, the information presented has been well digested and the data and conclusions of the data have been weighed carefully.

Algal metabolism is becoming of increasing importance as is evident from the increasing number of recent publications on this subject. From a theoretical point of view, the algae represent experiments in evolution. This is evident from a diversity of photosynthetic pigments in the various classes of algae and peculiarities of their products of metabolism. Therefore, they are of interest to the comparative biochemist and to the general physiologist as well. From the economic point of view, algae are also of importance. For example, the yield of fish in the oceans is directly dependent on algal growth. The oceans have an algal flora which carries on an amount of photosynthesis at least equivalent to that of land flora. If sufficient knowledge were available on algal biology and chemistry, it might be possible to increase the yield of algae in specific ocean areas and even to influence the yield of more desirable species. Algae may then come to play an increasingly important role in the extension of the world food supply.

For the specialist, this book on algal metabolism is not only valuable as a summary of recent advances of a newly developing subject but also because it provides a detailed bibliography. For the biology and biochemistry student, this book should also prove valuable as an introduction to the chemistry and metabolism of this diverse and fascinating group of organisms.

S. GRANICK

Fry, B. A. and J. L. Peel (Ed.), 1954. Autotrophic micro-organisms. 305 p., ill. $5.00. Cambridge University Press, New York.

Autotrophs are organisms which accumulate chemical energy from photochemical reactions or by the oxidation of inorganic substances, from which substances they also secure carbon and nitrogen for growth. Through food cycles all other forms of life are dependent upon them; but their importance is not only ecological and economic. Although their nutrition is simple, autotrophs have a greater range of biochemical accomplishment than any other living creatures. The analysis of their energy-yielding and synthetic reactions and the means by which the two are coupled has been most readily carried out with micro-organisms. Furthermore, the comparative biology of autotrophy depends upon a study of these forms where the diversity of the mechanisms already revealed is astonishing. In the relations between autotrophy and heterotrophy (derivation of matter and energy from the degradation of organic substances produced by autotrophs) lies the key to early stages in the evolution of life. *Autotrophic Micro-organisms* which consists of thirteen papers

given at the Fourth Symposium of the Society for General Microbiology held in London, April, 1954, demonstrates the growing awareness of this fascinating subject and treats it in a way that maintains the high standards of the three previous Symposia, held on other subjects.

F. J. RYAN

Fuller, Harry J., and Oswald Tippo, 1954 (revised). College botany. 993 p., ill. $6.90. Henry Holt and Company, New York.

George, John L., and Jean George, 1954. Bubo, the great horned owl. 184 p., illustrated by Jean George. $3.00. E. P. Dutton and Company, Inc. New York.

Haldane, J. B. S., 1954. The biochemistry of genetics. 144 pp., 15 shillings. George Allen and Unwin, London.

A series of lectures addressed to biochemists on principles and problems of genetics, illustrated by examples from biochemical genetics. Of greatest general interest are the two concluding chapters: "Mutation and the Problem of Gene Reproduction," and "Tentative Conclusions."

Howes, Paul Griswold, 1954. The giant cactus forest and its world. 258 p. 186 photographs, field sketches and diagrams; 1 color plate. $7.50. Duell, Sloan and Pearce, New York; Little, Brown and Company, Boston.

The curator of the Bruce Museum, Greenwich, Connecticut, records in popular style the results of numerous field trips to the giant cactus belt of Southern Arizona. This book will serve as a useful guide to the plants and animals for tourists and naturalists who visit this region.

Ingles, Lloyd Glenn, 1954. Mammals of California and its coastal waters. 396 p., ill. $6.00. Stanford University Press, Stanford, California.

Jahn, Raymond (editor), 1954. Tobacco dictionary. 199 p. $5.00. Philosophical Library, New York.

This book seeks to bring to the smoker the interesting, curious and necessary facts relating to the history, manufacture and use of tobacco.

Kent, George C., Jr., 1954. Comparative anatomy of the vertebrates. 530 p., 405 text-figures. $6.00. The Blakiston Company, Inc., New York.

Leeuwenhoek, Antoni van, 1954. The discovery of unicellular life. 14 p., gratis. Excerpts from communications by van Leeuwenhoek to the Royal Society of London, reprinted from his collected letters. Chronica Botanica Co., Waltham, Mass.

Leuba, Clarence, 1954. The natural man. 70 p., $.95, paper. Doubleday Papers in Psychology, Doubleday & Co., Inc., Garden City.